JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 56

JANUARY 1–JUNE 15, 1938

ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON: 1938

DARTMOUTH
COLLEGE
LIBRARY

630.61
U58j
v. 56

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, CHAIRMAN
Chief, Bureau of Chemistry and Soils

JOHN W. ROBERTS
Principal Pathologist, Bureau of Plant Industry

BENJAMIN SCHWARTZ
Principal Zoologist, Chief, Zoological Division, Bureau of Animal Industry

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
Director of Research, Pennsylvania Agricultural Experiment Station

L. E. CALL
Director, Kansas Agricultural Experiment Station

C. E. LADD
Director, New York (Cornell) Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:

Entire Journal: Domestic, $3.25 a year (2 volumes)
Foreign, $4.75 a year (2 volumes)
Single numbers: Domestic, 15 cents
Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

II

DEC 8 1938
425985
B.P.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 56 WASHINGTON, D. C. JANUARY 1–JUNE 15, 1938

CONTENTS

	Page
Nematodes Infesting Red Spiderlilies. Illus. G. STEINER	1
Initial Tests of the Distance of Spread to and Intensity of Infection on *Pinus monticola* by *Cronartium ribicola* From *Ribes lacustre* and *R. viscosissimum*. Illus. T. S. BUCHANAN and J. W. KIMMEY	9
Relation of the Curly Top Virus to the Vector, *Eutettix tenellus*. Illus. C. W. BENNETT and HUGH E. WALLACE	31
Influence of Certain Harmful Soil Constituents on Severity of Pythium Root Rot of Sugarcane. Illus. R. D. RANDS and ERNEST DOPP	53
The Vitamin A Value of Blue Grama Range Grass at Different Stages of Growth. MARGARET CAMMACK SMITH and E. B. STANLEY	69
Adaptation and Use of Automatically Operated Sand-Culture Equipment. Illus. H. D. CHAPMAN and GEORGE F. LIEBIG, Jr	73
Growth Prediction and Site Determination in Uneven-Aged Timber Stands. Illus. WILLIAM A. DUERR and S. R. GEVORKIANTZ	81
Relation of Certain Plant Characters to Strength of Straw and Lodging in Winter Wheat. Illus. I. M. ATKINS	99
Effect of Carbon Dioxide Storage on Bartlett Pears Under Simulated Transit Conditions. Illus. FISK GERHARDT and BOYCE D. EZELL	121
Use of the Dilatometer in Measuring the Extent of Freezing in Ice Cream and Related Products. Illus. WILFORD C. COLE	137
A Method for Extracting Growth Substances From Pigmented Tissues. Illus. H. G. DUBUY	155
Inheritance of Resistance to Loose and Covered Smuts in Markton Oat Hybrids. Illus. GEORGE M. REED and T. R. STANTON	159
Chromosomes of Maize From North American Indians. Illus. A. E. LONGLEY	177
Establishment and Spread of Molds and Bacteria on Cotton Roots by Seed and Seedling Inoculation. Illus. MARIE B. MORROW, JAMES L. ROBERTS, JAMES E. ADAMS, H. V. JORDAN, and PAUL GUEST	197
The Comparative Value of Cod-Liver Oil Alone and in Combination With Irradiated Ergosterol as a Source of Vitamin D for Confined Laying Hens. RALPH B. NESTLER	209
Resistance of Chicken Embryos to Mechanical Disturbances. Illus. MARLOW W. OLSEN and THEODORE C. BYERLY	221
The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single-Feeding Method. Illus. M. M. KRAMER, MARGARET D. BAIR, BERNICE L. KUNERTH, and W. H. RIDDELL	227
Vitamin G (Riboflavin) Content of Colostrum and Milk of Cows Determined Biologically. Illus. M. M. KRAMER, INEZ GARDNER, BERNICE L. KUNERTH, and W. H. RIDDELL	233
Life Span and Morphology of Fire Blight Bacteria as Influenced by Relative Humidity, Temperature, and Nutrition. Illus. H. R. ROSEN	239
Factors Influencing the Discovery of Forest Fires by Lookout Observers. Illus. CHARLES C. BUCK	259
Mechanographic Method of Recording Insect Cardiac Activity, With Reference to Effect of Nicotine on Isolated Heart Preparations of *Periplaneta americana*. Illus. J. FRANKLIN YEAGER	267
The Toxicity of Phenothiazine and Some of Its Oxidation Products, in Experiments With *Carassius auratus*. Illus. W. A. GERSDORFF and H. B. CLABORN	277
A Portable Instrument for the Analysis of Hydrocyanic Acid Gas-Air Mixtures. Illus. J. R. WILMOT and T. N. GAUTIER	283
Fungus Growth in Shelled Corn as Affected by Moisture. Illus. BENJAMIN KOEHLER	291

	Page
Some Correlations in Plant-Tissue Composition, Decomposition Products, and Effect Upon Crop Rotation With Tobacco. WALTER S. EISENMENGER	309
Two Fungi Causing Leaf Spot of Peanut. Illus. WILBERT A. JENKINS	317
Hopi Cotton, a Variable Species. Illus. H. J. FULTON	333
Oxidase and Catalase Activity of Bartlett Pears in Relation to Maturity and Storage. Illus. BOYCE D. EZELL and FISK GERHARDT	337
A Comparative Study of an Early, a Medium, and a Late Strain of Timothy Harvested at Various Stages of Development. MORGAN W. EVANS and LLOYD E. THATCHER	347
Respiration and Oxidase and Catalase Activity of Apple and Pear Fruits. Illus. BOYCE D. EZELL and FISK GERHARDT	365
The Influence of Various Factors on Egg Production in Turkeys. V. S. ASMUNDSON	387
A Morphological Study of Flower and Seed Development in Pepper. Illus. H. L. COCHRAN	395
Studies on Longevity and Productivity in *Trichogramma evanescens*. Illus. HORACE O. LUND	421
Meiotic Instability as an Inherited Character in Varieties of *Triticum aestivum*. W. M. MYERS and LEROY POWERS	441
Quality Studies in the Wheat-Breeding Program at the Minnesota Agricultural Experiment Station. E. R. AUSEMUS, M. C. MARKLEY, C. H. BAILEY, and H. K. HAYES	453
Correlation of Body Measurements of Slaughter Steers With Rate and Efficiency of Gain and With Certain Carcass Characteristics. Illus. W. H. BLACK, BRADFORD KNAPP, Jr., and A. C. COOK	465
Spotting of Figs on the Market. Illus. CHARLES BROOKS and L. P. MCCOLLOCH	473
Effect of Low Concentrations of Copper on Germination and Growth of Conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Illus. M. C. GOLDSWORTHY and E. L. GREEN	489
The Origin of Lint and Fuzz Hairs of Cotton. Illus. A. G. LANG	507
Rogas unicolor (Wesm.), a Braconid Parasite of the Satin Moth. PHILIP B. DOWDEN	523
Transmission of Sugarcane Mosaic by the Green Bug (*Toxoptera graminum* Rond.) J. W. INGRAM and E. M. SUMMERS	537
A Mosaic Disease of Chinese Cabbage. Illus. C. M. TOMPKINS and H. REX THOMAS	541
Evaluation of Some Soil Fungicides by Laboratory Tests With *Phymatotrichum omnivorum*. Illus. WALTER N. EZEKIEL	553
Tests With Pentachlorethane, Tetrachlorethane, and Xylol to Determine Their Efficiency in Eradication of Phymatotrichum Root Rot. Illus. WALTER N. EZEKIEL	579
A Cytological Study of Host-Parasite Relations of *Venturia inaequalis* on Apple Leaves. Illus. CHARLES J. NUSBAUM and G. W. KEITT	595
Determining the Sclerotial Population of *Sclerotium rolfsii* by Soil Analysis and Predicting Losses of Sugar Beets on the Basis of These Analyses. Illus. L. D. LEACH and A. E. DAVEY	619
The Effects of Temperature and Other Environmental Factors Upon the Photoperiodic Responses of Some of the Higher Plants. Illus. R. H. ROBERTS and B. ESTHER STRUCKMEYER	633
Studies on the Physiology and Inheritance of Feathering in the Growing Chick. M. H. RADI and D. C. WARREN	679
Sorghum Characters Grouped by Multiple Correlations. J. C. IRELAND	707
Directional Permeability of Seasoned Woods to Water and Some Factors Which Affect It. HARVEY D. ERICKSON, HENRY SCHMITZ, and R. A. GORTNER	711
A Streak Disease of Peas and Its Relation to Several Strains of Alfalfa Mosaic Virus. Illus. W. J. ZAUMEYER	747
Studies on the Cause of Immunity of Monocotyledonous Plants to Phymatotrichum Root Rot. Illus. WALTER N. EZEKIEL and J. F. FUDGE	773
A Measure of Toxicity in Plant Studies. Illus. PHILIP L. GILE	787
Effect of Percentage of Protein in the Diet on Growth and Feed Utilization of Male Chickens. Illus. JOHN C. HAMMOND, WALTER A. HENDRICKS, and HARRY W. TITUS	791

Page

Energy Requirements of Swine and Estimates of Heat Production and Gaseous Exchange for Use in Planning the Ventilation of Hog Houses. Illus. H. H. MITCHELL and M. A. R. KELLEY 811

Toxicity of Selenium Fed to Swine in the Form of Sodium Selenite. Illus. W. T. MILLER and H. W. SCHOENING 831

The Measurement and Inheritance of Scab Resistance in Selfed and Hybrid Progenies of Potatoes. Illus. J. G. LEACH, F. A. KRANTZ, PHARES DECKER, and HAROLD MATTSON 843

The Control of Dandelions in Lawns. Illus. W. E. LOOMIS 855

Influence of Certain Environmental Conditions on Congestion of Starch in Tomato Plant Stems. Illus. ARTHUR C. FOSTER and EVERETT C. TATMAN 869

Studies of Selected Strains of Curly Top Virus. Illus. N. J. GIDDINGS 883

Mosaic of Lima Beans (*Phaseolus lunatus macrocarpus*). Illus. L. L. HARTER 895

Sand Dunes of Recent Origin in the Southern Great Plains. Illus. CHARLES J. WHITFIELD 907

The Effect of the X Factor of Sodium Chloride and of the Composition of the Nutrient Media Upon the Growth of the Fowl Coryza Bacillus, *Hemophilus gallinarum*. J. P. DELAPLANE, L. E. ERWIN, and H. O. STUART 919

A Critical Evaluation of the Rat-Growth Method for Determining Vitamin B and Its Content in Meals From Certain Oily Seeds. Illus. F. W. SHERWOOD and J. O. HALVERSON 927

V561 JAN 1 38 XUM

AUTHORS' ERRATA AND EMENDATIONS

Page 6, sixteenth line from bottom, insert "to 29.3" after "$\alpha = 22.4$,"
Page 16, footnote 2, table 3, Reference (*10*) should be Reference (*9*).
Page 30, reference (8a), "1938" should be "1937."
Page 159, first paragraph, line 8, "(*Ustilago avenae*) (Pers. Jens.)" should be "(*Ustilago avenae* (Pers.) Jens.)"
Page 162, figure 1, "50–c–F–229–2" should be "50–C–F_1–229–2."
Page 180, table 1, last two columns, delete the word "knobs" in the boxheads.
Page 198, eleventh line from bottom, "treatment 2,4" should be "treatment $2,^3$."
Page 205, sixth line from bottom, "six" should be "eight."
Page 228, table 1, last column, first line, "7.27" should be "17.27."
Page 273, sixth line from bottom, "Kojantchikov's lever made contact with the heart by means of a piece" should read "Kojantchikov contacted the heart with a piece."
Page 288, "*p*" should be "*n*."
Page 331, last word on page, "Arachi-" should be "Arachis."
Page 352, table 3, first column, "1933–34" should be "1932–34."
Page 383, line 11, "decompose hydrogen peroxide in oxygen and water" should be "decompose hydrogen peroxide into oxygen and water."
Page 390, first line, "table 1" should be "table 2."
Page 427, ninth line from bottom, a shilling mark (/) should be inserted between "difference" and "S. E."
Page 458, table 2, second column, "58" should be in boldface type.
Page 492, line 18, "per million" should be "per milliliter."
Page 738, line 6, omit "contrary" between the words "the" and "was."
Page 758, first and third paragraphs, "μ" should be "mm" wherever used.
Page 802, table 6, last two columns, the headings should be so arranged that they will read: "C divided by percentage of protein" and "Standard error of C divided by percentage of protein."
Page 832, table 1, last column, sixth figure from bottom, "−2.5" should be "+2.5."
Page 834, table 3, the sentence immediately under the heading, "Results given in terms of per million of selenium parts" should read "Results given in terms of parts of selenium per million."
Page 888, table 2, under "Susceptible Beets," column 6, "684.1" should be "648.1."
Page 888, table 3, last line, next to last column, "1.1" should be "1.0."
Page 889, table 4, first line, last column, should read minus instead of plus.
Page 892, table 5, under "Turkish Tobacco," "Mar. 3, 1936" should be "Mar. 23, 1936."
Page 928, table 1, column five, third line, ".20" should be ".21."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 56 WASHINGTON, D. C., JANUARY 1, 1938 NO. 1

NEMATODES INFESTING RED SPIDERLILIES[1]

By G. STEINER[2]

Principal nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In various lots of diseased red spiderlilies (*Lycoris radiata* Herb.),[3] submitted in recent years to the Division of Nematology, several parasitic nematode species have been observed. One lot of red spiderlilies, grown near Hamlet, N. C., and submitted in January 1933 by R. W. Leiby, State entomologist, Raleigh, N. C., harbored the bulb or stem nematode, *Ditylenchus dipsaci* (Kühn, 1858) Filipjev, 1936, in the bulbs and leaves, and an undescribed species of *Rotylenchus* in the roots. Three lots were submitted by N. R. Hunt, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. The first of these, from Castle Hayne, N. C., contained the two nemic species just mentioned. The second, originating from Westminster, S. C., was infested by the bud or leaf nematode, *Aphelenchoides fragariae* (Ritzema Bos, 1891) Christie, 1932. The third, from Peachland, N. C., contained the above-named *D. dipsaci*. A fifth lot, from Lexington, S. C., submitted by G. M. Armstrong, of Clemson Agricultural College, had roots infested by the *Rotylenchus* mentioned above.

DITYLENCHUS DIPSACI AS A PARASITE IN THE BULBS AND LEAVES OF THE RED SPIDERLILY

The former *Anguillulina dipsaci* (Kühn, 1858) Gerv. and v. Ben., 1859, was recently made the type species of a new genus, *Ditylenchus*.[4] The name *Ditylenchus dipsaci* is therefore used here as the new scientific term for the well-known bulb or stem nematode. Of the two occurrences of this form in the red spiderlily (for general appearance of this plant, see fig. 1), the one from near Hamlet, N. C., is of special interest, because in this case the infestation probably originated from infested narcissus bulbs growing next to the red spiderlily. Few cases have been observed of such apparent natural transfer of the bulb or stem nematode from one host to another. The close botanical relationship and the similarity of the bulb structure of the red spiderlily to that of the narcissus may account for such a transfer. The disease symptoms in the red spiderlily bulb, which are shown in figure 2, *A*, very much resemble those produced by the same parasite in narcissus bulbs. Of 40 red spiderlily plants examined from this

[1] Received for publication October 9, 1937; issued March 1938.

[2] The author is indebted to Mrs. Florence Albin, of the Division of Nematology, who assisted in the preparation of the text figures, and to E. Artschwager, of the Division of Sugar Plant Investigations, Bureau of Plant Industry, who aided in the interpretation of the root tissues.

[3] This material had been submitted under the name nerine lily (*Nerine sarniensis* Herb.). B. Y. Morrison and S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, however, determined the host plant to be the red spiderlily (*Lycoris radiata* Herb.). The nerine lily is therefore wrongly recorded as a host of *Ditylenchus dipsaci* (Kühn) Filipjev (see: STEINER, G., and BUHRER, E. M. NOTES ON NEMIC DISEASES. U. S. Dept. Agr. Plant Disease Reptr. 16: 169. 1932. [Mimeographed]).

[4] FILIPJEV, I. N. ON THE CLASSIFICATION OF THE TYLENCHINAE. Helminthol. Soc. Wash., Proc. 3: 80-82. 1936.

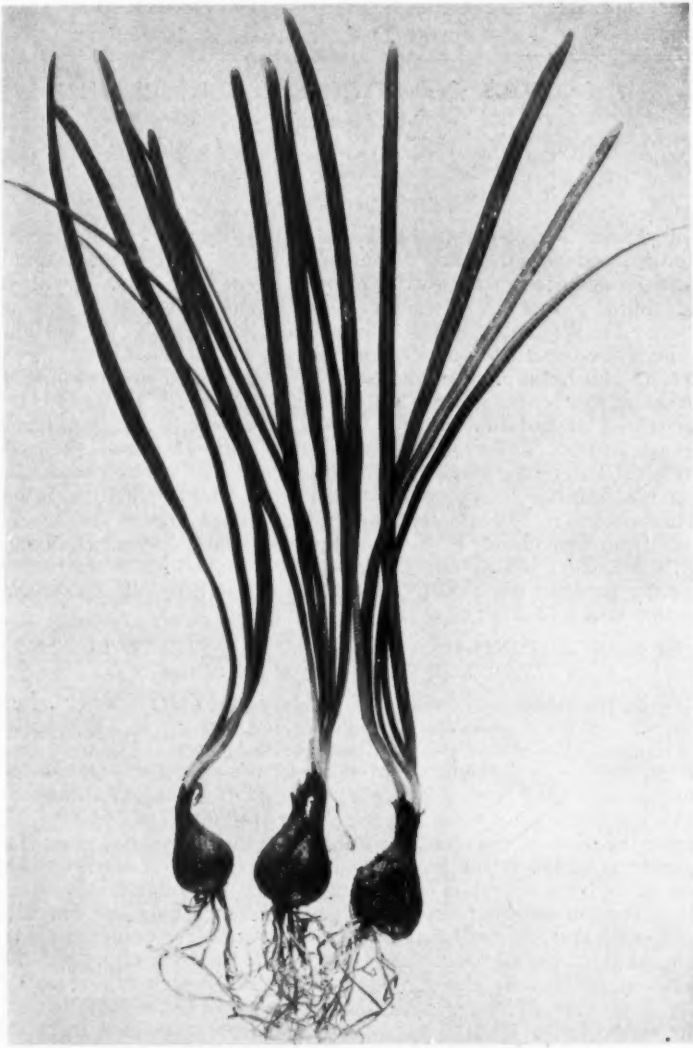

FIGURE 1.—Red spiderlilies from Hamlet, N. C., with roots infested by *Rotylenchus brachyurus* n. sp. × ⅓.

location, however, only 2 harbored the bulb or stem nematode, whereas the *Rotylenchus* species was present in all of them.

The "brown rings" are not so clear-cut in the red spiderlily bulbs as in the narcissus bulbs, and the infection apparently spreads more in a radial direction. During the growing season the nemas are also found in great numbers in some of the leaves (fig. 2, *B*), but here the reaction of the red spiderlily as host is quite different from that of the narcissus. Observations extending over a period of 3 years did

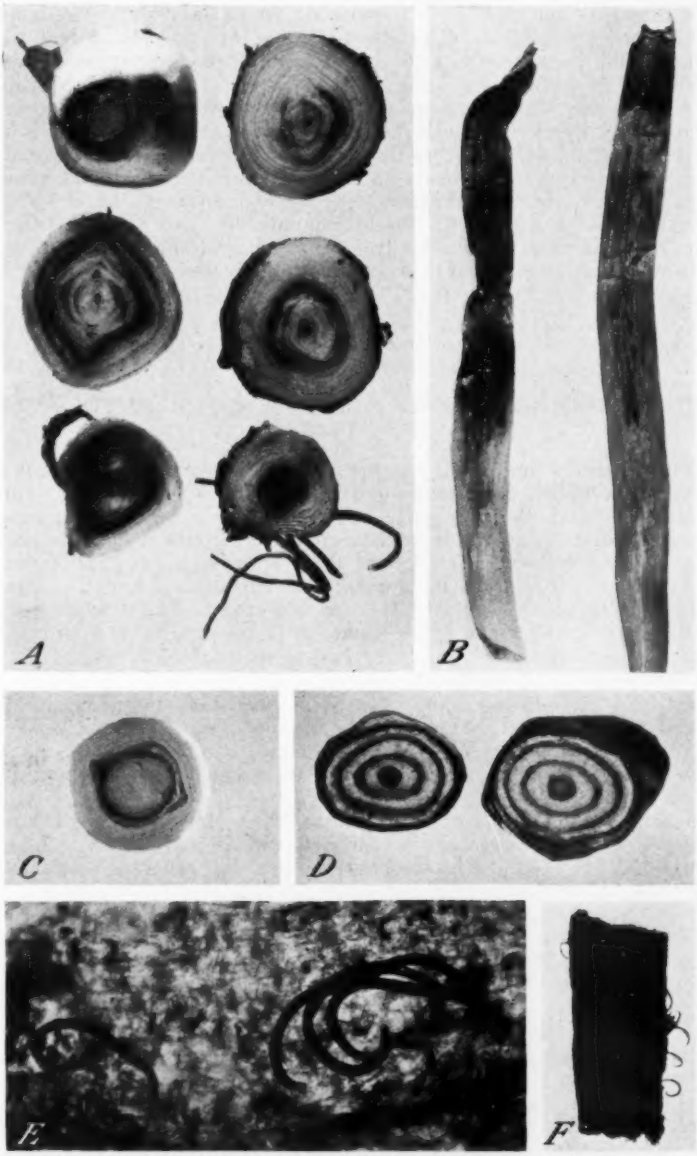

FIGURE 2.—*A*, Red spiderlily bulbs infested with the bulb or stem nematode, *Ditylenchus dipsaci*. Cross sections through two different bulbs; sections through each bulb in vertical arrangement; neck region at top; basal region at bottom. × ¾. *B*, Red spiderlily leaves infested with the bulb or stem nematode, *Ditylenchus dipsaci*. Actual size. *C*, Cross section through red spiderlily bulb infested with the bud or leaf nematode, *Aphelenchoides fragariae*. × ¾. *D*, Cross sections through red spiderlily bulb infested with *Cephalobus persegnis*. × ¾. *E*, Photomicrograph of *Rotylenchus brachyurus* specimens on roots of the red spiderlily. × 65. *F*, Profile view of a root portion of the red spiderlily with *Rotylenchus brachyurus* specimens attached to the surface. × 14.

not disclose "spickles" or otherwise deformed leaves in greenhouse-grown red spiderlilies. The affected areas of an infested leaf turn yellow and then brown, and the leaf finally collapses and decays. As far as observed, this disease is not so virulent in red spiderlilies as it is in narcissus. It develops rather gradually over a period of a year or more but eventually kills the plant. It spreads very slowly to new plants. On the other hand, the nematodes were able to maintain themselves in these hosts under environmental conditions (greenhouse during the hot Washington, D. C., summer) under which narcissus bulbs have been found regularly to lose their parasites. The dimensions of specimens from the red spiderlily bulbs are well within the known range of the species, although close to the lower limit, the size being rather small. The measurements are as follows:

♀: Total length=1.2 to 1.3 mm; α=36. to 51., β=6.7 to 7.8, γ=15.8 to 21., V=80 to 84 percent; distance vulva to anus, about twice length of tail.
♂: Total length=1.1 to 1.3 mm; α=48. to 57.6, β=7. to 8.8, γ=17. to 18.2.

ROTYLENCHUS BRACHYURUS N. SP. AS A ROOT PARASITE OF THE RED SPIDERLILY

There are few published records concerning the plant-pathological significance of the hoplolaims and related forms such as the tylenchorhynchs and the rotylenchs. This group, however, is of much more significance in plant pathology than records would indicate. The writer possesses data showing that representatives of this group are especially numerous in tropical and subtropical regions, where many species affect a variety of crops and trees. The new species of *Rotylenchus* is undoubtedly a parasite of the root (fig. 1); in only one instance were specimens seen attached to the surface of a bulb of the red spiderlily. Unlike the heteroderas, the new species is of the vagrant type of plant-parasitic nematodes, apparently migrating in and out of a root, often feeding from the outside (figs. 2, *F*; 3, *C*). Frequently whole nests of specimens are found, manifestly always in the root exodermis (fig. 3, *A*, *B*), feeding on the cell contents, breaking up the tissue, and opening it for decay.

Specimens have not been observed to penetrate into a root beyond the exodermis. The reasons for this behavior are not known. Suberized cells, which might form a barrier, are apparently absent from the tissue from the exodermis inward. Thickened cell walls delimit the exodermis on its surface (fig. 3, *A*), but they were evidently no hindrance to penetration by the nemas. It may be remarked, however, that in younger roots, with the epidermis still present, these thickened cell walls are seen only at intervals (fig. 3, *B*), the nemas having therefore ample opportunity to penetrate between them. Even so, it is thought that such thickened walls would not form a mechanical obstacle to a species with a buccal stylet of such strength as that of the new species of *Rotylenchus*. The damage caused by these rotylenchs in their hosts is doubtless of less significance than that produced by the heteroderas and similar forms. The rotylenchs attack only the exodermis, and it appears that the host may withstand such injury to a high degree although the production of flowers may be checked to some extent.

Eggs are deposited within the exodermal tissues, apparently in an unsegmented stage, the larvae developing within the plant tissues. That this species leads an exclusively parasitic mode of life is also

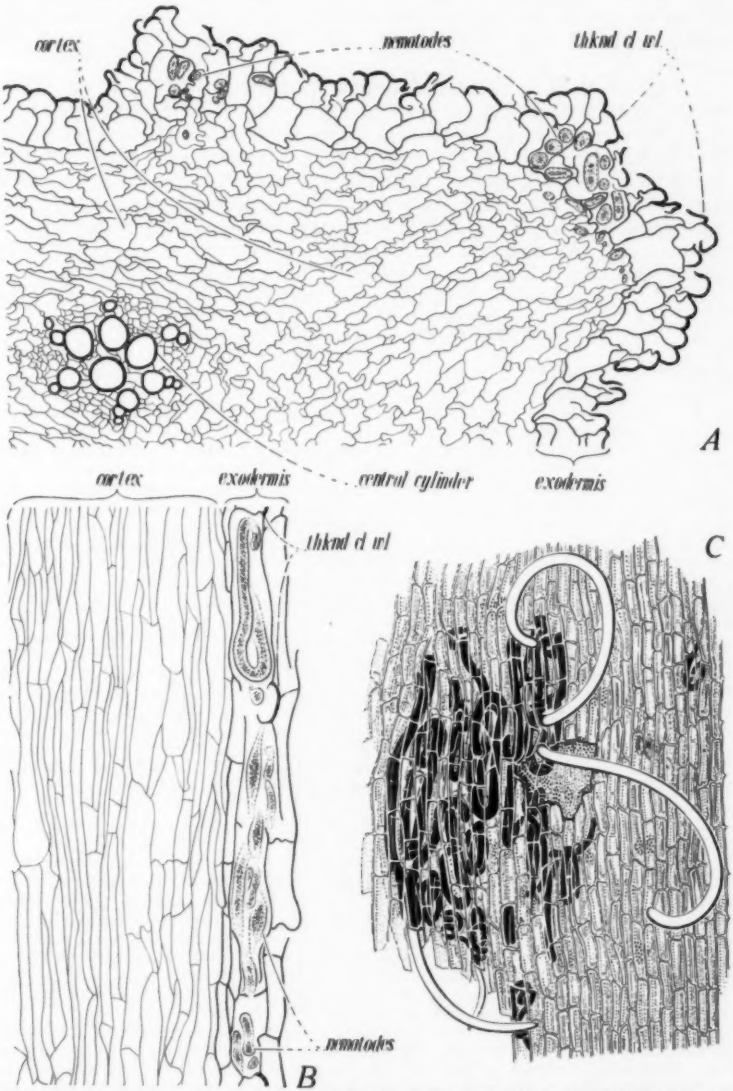

FIGURE 3.—*A*, Part of a cross section through a root of a red spiderlily with nematodes (*Rotylenchus brachyurus* n. sp.) in the exodermis; *thknd cl wl*, thickened walls of cells originally separating the epidermal cell layer (which has disappeared) from the exodermis. × 120. *B*, Longitudinal (tangential) section through part of an infested root of a red spiderlily; nematodes are again found only in the exodermis; *thknd cl wl*, thickened walls of cells, here separated by long intervals. × 120. *C*, Surface view of portion of red spiderlily root with a nest of nematodes (*R. brachyurus* n. sp.), some hanging on the outside with only the head portion inside the tissue. × 75.

suggested by the frailty of the rectum, the apparent reduction of the intestinal cavity, and the opacity of the intestine itself, which is filled with reserve material that makes it appear dark. Although

several hundred specimens were examined, no males were observed, which suggests that the species is either hermaphroditic or parthenogenetic.

In figure 4, *B*, a sketch of the molting head end is given, which demonstrates that here, as in other tylenchs, only the anterior, i. e., the conical portion of the buccal stylet is shed, including the wall of the buccal cavity with its thickened portions to the same distance posteriad. The whole cuticularized framework with its processes is part of the cuticle. The fact that the cylindrical portion and the basal knobs of the stylet are often indistinct in molting specimens, in contrast to the distinctness of the anterior conical part, deserves special mention. These two portions of the stylet are manifestly not only of different origin (ectoderm vs. entoderm) but also of different substance.

TECHNICAL DESCRIPTION

Rotylenchus brachyurus n. sp.

Body usually sickle-shaped or curved in form of loose spiral (figs. 2, *E*; 3, *C*; 4, *K*), cylindrical; tail end broad, even concave (fig. 4, *F*), obtuse; anterior end tapering cephalad of intestine.

Cuticular annulation often rather obscure except on tail end, where distinct. Annuli about 1.5μ wide, laterally interrupted by three longitudinal bands representing the lateral fields. They begin in middle region of unprotruded buccal stylet (fig. 4, *H*), are subdivided by transverse striae in esophageal region (fig. 4, *H*, *I*), then plain (fig. 4, *J*), and end close to tail terminus (fig. 4, *E*, *G*). Annulation on tail following contour of end (fig. 4, *E*, *G*). Scutellumlike phasmid circular to oval, located in latitude of, or slightly posterior to, anus. Lateral bands, anteriorly (fig. 4, *H*) beginning as one, separating into two and then three just in front of middle esophageal bulb, then continuing to tail end (fig. 4, *E*, *G*, *I*, *J*).

Head well set off, conoid obtuse, with five annuli and heavily cuticularized framework (fig. 4, *D*). First annulus behind head, with somewhat heavier cuticularization (fig. 4, *A*, *B*). Buccal stylet strong, about 29μ long, with almost spherical basal knobs, which are only slightly flattened anteriorly; just back of head a distinct conical guiding ring for this stylet (fig. 4, *A*, *B*). Esophagus typical; isthmus and terminal portion very slender, esophageal glands overlapping intestine; end of esophagus set off from intestine, with vestigial, exceedingly small valves (fig. 4, *C*). Intestine opaque, cell walls obscure, cells filled with globular reserve material. Rectum indistinct, almos tobscure; anus only about 10 annuli from tail end.

Excretory pore always in latitude of last esophageal gland, i. e., behind beginning of intestine.

Female sexual apparatus amphidelphic (fig. 4, *K*), anterior as well as posterior branch to left of intestine, each branch about 22 to 25 percent of total body length. Ovaries outstretched, narrow, consisting of few cells in single series. Oviduct very short, uterus with only one egg at a time. Eggs apparently deposited unsegmented. Vagina with thickened walls, leading straight inward. Opacity of intestine often renders close study of female apparatus difficult. Propagation apparently hermaphroditic or parthenogenetic.

Measurements.— ♀: Total length=0.72 to 0.89 mm; α=22.4; β=5.6 to 6.4; γ=47.to 80.; V=59 to 61 percent; eggs=24μ×68μ (fig. 4, *L*).

Diagnosis.—Apparently hermaphroditic or parthenogenetic *Rotylenchus;* cuticle differentiated on lateral fields into three longitudinal bands, two outer ones beginning in region of buccal stylet, separating into three just anterior to middle esophageal bulb, and then continuing past phasmid close to tail end; esophageal portion of these bands areolated by transverse striae; these correlated to annulation of cuticle. Scutellum-shaped phasmid oblong to circular, in latitude of anus. Annulation following contour of tail end. Tail short, broad obtuse, end sometimes concave, its length one-half to three-fourths of anal body diameter. Head with five annuli and strong cuticularized framework. Buccal stylet strong, with spherical, anteriorly slightly flattened but not indented basal knobs. Wall of vestibulum surrounding conical portion of stylet thickened, thus forming a guiding ring

Type locality.—North Carolina.

Type host.—*Lycoris radiata* Herb.

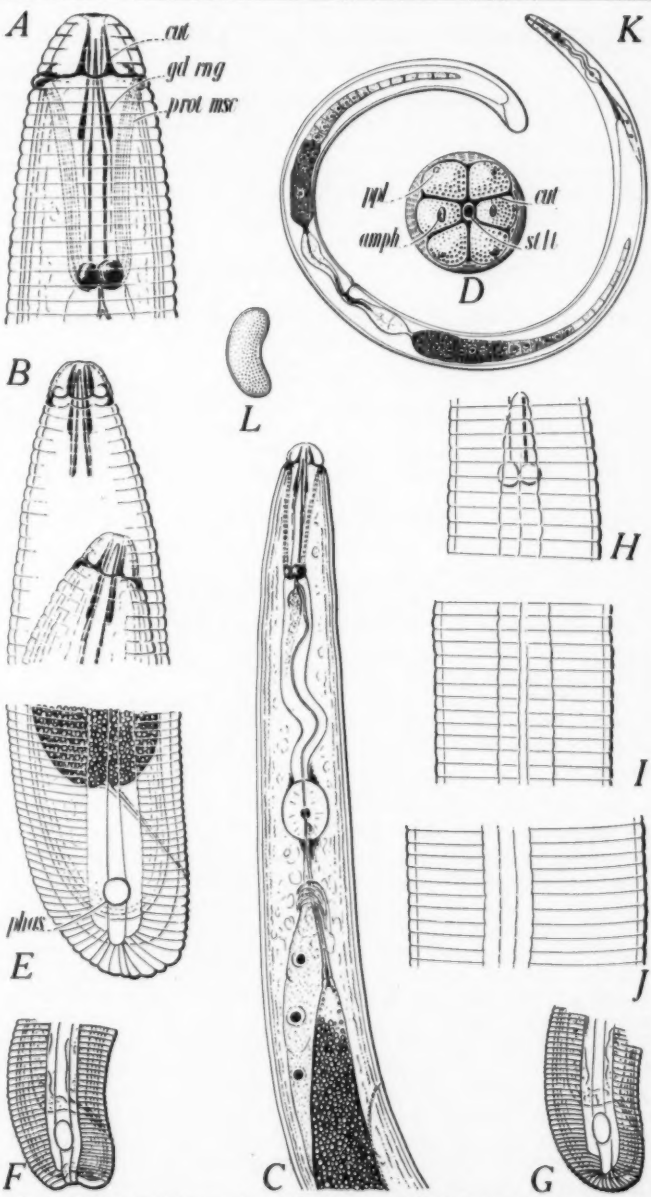

FIGURE 4.—*Rotylenchus brachyurus* n. sp. *A*, Head end: *cut*, cuticular framework; *gd rng*, guiding ring; *prot msc*, protractor muscle of buccal stylet. ×1,750. *B*, Molting head end. ×1,250. *C*, Anterior end of body. ×665. *D*, Front view of head: *amph*, amphid; *ppl*, cephalic papilla; *cut*, cuticular framework; *stlt*, stylet. ×1,710. *E*, Tail end of female: *phas*, phasmidial scutellum. ×1,250. *F*, Tail end of female with slightly concave terminus. ×665. *G*, Tail end of female, another variation. ×665. *H*, Detail of anterior end of lateral bands. ×1,250. *I*, Detail of lateral bands in region of esophageal isthmus. ×1,250. *J*, Detail of lateral bands in postesophageal region. ×1,250. *K*, View of female sexual apparatus in its position in the body. ×200. *L*, Egg. ×200.

APHELENCHOIDES FRAGARIAE AS A PARASITE OF THE RED SPIDERLILY BULB

Figure 2, *C*, represents a cross section through a red spiderlily bulb parasitized by the bud or leaf nematode, *Aphelenchoides fragariae*. The disease symptoms produced in the bulb are also of the "brown ring" type and resemble those produced by *Ditylenchus dipsaci* in red spiderlily bulbs and those so well known in narcissus bulbs. The dimensions of the present specimens agree well with the type; measurements of a female were as follows:

♀: Total length=0.76 mm; $a=51$; $\beta=11$; $\gamma=16.8$; V=67 percent.

CEPHALOBUS PERSEGNIS CAUSING BROWN RING SYMPTOMS ON THE RED SPIDERLILY BULB

In the lot of 40 red spiderlily bulbs referred to above as infested with *Rotylenchus brachyurus*, there was a single bulb which, besides being affected by *R. brachyurus*, on being cross-sectioned exhibited ring symptoms as shown in figure 2, *D*. These proved to harbor numerous specimens of *Cephalobus persegnis* Bastian, 1865.

DORYLAIMUS SUBTILIS AS A ROOT PARASITE OF THE RED SPIDERLILY

Dorylaimus subtilis Thorne and Swanger, 1936, has been previously recorded only from the State of Utah.[5] The finding of rather numerous specimens in roots of the red spiderlily from Hamlet, N. C., suggests, if not a parasitic, at least a semiparasitic, mode of life in plant roots. In male specimens there were six to eight so-called supplements, i. e., series of ventromedian preanal papillae, separated from the adanal papilla by about the length of the spiculum. This species was also observed in roots of the narcissus, variety Olympia, grown in Castle Hayne, N. C.

SUMMARY

Red spiderlilies (*Lycoris radiata* Herb.) in the South Atlantic region of the United States are subject to the attacks of the following plant-parasitic nematodes: *Ditylenchus dipsaci* (Kühn) Filipjev, the bulb or stem nematode; *Aphelenchoides fragariae* (Ritzema Bos) Christie, the bud or leaf nematode; and *Rotylenchus brachyurus* n. sp., a new entoparasite of the vagrant type, attacking the root exodermis exclusively. In one instance, the infestation by the bulb or stem nematode is considered as having originated from narcissus by an apparent natural transfer from that host. The symptoms caused by *D. dipsaci* and *A. fragariae* in the red spiderlily bulb are brownish rings similar to those produced by these parasites in narcissus bulbs. *Cephalobus persegnis* Bastian likewise was found as the apparent cause of brown rings in red spiderlily bulbs, although experimental proof of this relationship is lacking. *Dorylaimus subtilis* Thorne and Swanger was also found as a root parasite but is obviously of little significance as a disease agent.

[5] THORNE, G., and SWANGER, H. H. A MONOGRAPH OF THE NEMATODE GENERA DORYLAIMUS DUJARDIN, APORCELAIMUS N. G., DORYLAIMOIDES N. G. AND PUNGENTUS N. G. Capita Zoologica v. 6, pt. 4, 233 pp., illus. The Hague. 1936.

INITIAL TESTS OF THE DISTANCE OF SPREAD TO AND INTENSITY OF INFECTION ON PINUS MONTICOLA BY CRONARTIUM RIBICOLA FROM RIBES LACUSTRE AND R. VISCOSISSIMUM [1]

By T. S. BUCHANAN and J. W. KIMMEY, *assistant pathologists, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

The discovery of white-pine blister rust (*Cronartium ribicola* Fisch.) infections in British Columbia, Canada, in 1921 (*5*) [3] and the extension of the known range as determined in 1922 [4] pointed to the ultimate discovery of the disease in the valuable western white pine (*Pinus monticola* Dougl.) stands of northern Idaho, western Montana, and northeastern Washington, particularly in northern Idaho, adjacent to the international boundary. Preliminary control measures were instituted in the West in 1922.[5] Following the general procedure employed in the East, where blister rust control was already in operation, the Office of Blister Rust Control [6] of the Bureau of Plant Industry confined its initial efforts chiefly to enforcement of plant quarantine regulations, eradication of the highly susceptible European black currant (*Ribes nigrum* L.),[7] and experimental eradication of native forest ribes [8 9 10] (*13*). During the same year the Office of Forest Pathology,[11] Bureau of Plant Industry, initiated various studies on the life history and characteristics of the causal organism and the reaction of its hosts.

In the spring of 1928 blister rust was found on western white pine at Newman Lake, Wash., and in the fall of the same year it was noted in Bonner, Idaho, and in Shoshone and Clearwater Counties in northern Idaho, but on ribes only. In 1929 it was found on pines in Clearwater and Shoshone Counties (*13*). These discoveries showed conclusively that blister rust had, as anticipated, invaded the white pine area, and by 1930 local control, as planned in the large-scale blister rust control program (*1*, *3*), was under way.

[1] Received for publication April 2, 1937; issued March 1938.

[2] The authors are indebted to G. B. Posey, formerly of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, who first pointed out the desirability of making these tests, and to J. L. Mielke, of the Division of Forest Pathology, and H. G. Lachmund, formerly of the same Division, for the initiation of this study. W. F. Cummins and A. A. McCready, formerly of the same Division, rendered valuable assistance in the collection of the basic data. Acknowledgments are also extended to Dr. H. T. Güssow, of the Dominion of Canada Department of Botany, and to G. P. Melrose, of the British Columbia Forest Branch, for their friendly cooperation in the work carried out in British Columbia.

[3] Reference is made by number (italic) to Literature Cited, p. 29.

[4] DAVIDSON, A. T. WHITE PINE BLISTER RUST IN BRITISH COLUMBIA. (Report on Canadian conditions and work.) 3rd Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 9–22. [Mimeographed.]

[5] POSEY, G. B. REPORT OF WORK PERFORMED BY WESTERN OFFICE OF BLISTER RUST CONTROL. 3rd Western White Pine Blister Rust Conf., Portland, Oreg., 1922, Rept. Proc., pp. 5–9. [Mimeographed.]

[6] Now the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine.

[7] POSEY, G. B. See footnote 5.

[8] The common noun "ribes" is used to include species of both *Ribes* and *Grossularia*.

[9] WYCKOFF, S. N. REPORT OF WORK OF THE WESTERN BRANCH, OFFICE OF BLISTER RUST CONTROL, SEASON OF 1924. Western White Pine Blister Rust Conf., meeting of trustees, Seattle, Wash., 1924, Rept. Proc., pp. 6–8. [Mimeographed.]

[10] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. REPORT OF THE WESTERN BRANCH, OFFICE OF BLISTER RUST CONTROL, TO THE EXECUTIVE COMMITTEE OF THE WESTERN WHITE PINE BLISTER RUST CONFERENCE FOR THE PERIOD APRIL 1 TO NOVEMBER 30, 1923. 20 pp. [Mimeographed.]

[11] Now the Division of Forest Pathology.

Surveys and studies conducted by the Office of Blister Rust Control and by the Office of Forest Pathology indicated that the stream bottoms were the most imminent sources of danger because of the combination therein of concentrations of the most susceptible native ribes with the most favorable climatic conditions for intensification of the rust (*13*). Consequently control effort was directed primarily to the stream types, to which the highly susceptible wild black currant (*Ribes petiolare* Dougl.) and white-stemmed gooseberry (*R. inerme* Rydb.) are confined, and where the less susceptible but numerically important prickly currant (*R. lacustre* (Pers.) Poir.) also occurs (*9*, *13*).

The place in the control program of the upland types, characterized by abundant and widely scattered prickly currant and sticky currant (*Ribes viscosissimum* Pursh) (*13*), was not so clear. Tests of these two species had shown them to be relatively low in susceptibility, with the prickly currant apparently the less menacing (*9*). In spite of the low susceptibility of these two species, their wide range in habitat and consequent close association with stands of western white pine made their consideration in the control program imperative. That dense concentrations of these two species should be eradicated was fairly obvious, but where they occurred scattered over large areas the need for their partial or complete eradication was doubtful. In an attempt to secure more definite information on the subject, the Office of Forest Pathology found it possible to begin field work in British Columbia in 1928 on so-called ribes-to-pine spread plots wherein the capacity of the prickly currant and the sticky currant for spreading damaging infection to western white pine would be tested. Similar studies are now being conducted in Idaho, where the results obtained will be directly applicable to the conditions existing in the white pine stands of that State. The present paper reports the results of the investigations that were carried out in British Columbia.

EXPERIMENTAL PLOTS

SELECTION AND PREPARATION

The ideal conditions for the study would have been afforded by a series of areas wherein only one bush or one compact group of bushes of the desired ribes species existed at the center of a group of uninfected pines of uniform distribution. If this central bush should have become infected and spread infection to the surrounding pines, then all resultant pine infection would have been directly traceable to that bush, and if the amount of telia produced were known a measure would have been secured of the capacity of that species for spreading infection under natural conditions. To find a series of such areas was obviously not feasible, leaving as the best alternative the following approximation of the ideal.

Areas of uninfected western white pine of such a size as to permit their ready and thorough examination were located. On each selected area all ribes present within a block 990 feet square (22.5 acres), except those left at the centers to be inoculated, were eradicated as a precaution against extraneous pine infection. Periodic check eradications prevented any reestablishments. As a further precaution all ribes concentrations within a strip approximately 330 feet wide around these areas and, when necessary, within one-half

mile of the area boundaries, were removed. If native ribes did not occur at a desired center, native bushes were transplanted to form the center. These central ribes bushes, of known footage of live stem, were then inoculated in the spring of the year by dusting them with viable aeciospores immediately preceding a rain. Periodically throughout the season the resultant telia production was recorded. On some of the areas two successive tests were conducted (1928 and 1930), 1 year being allowed to elapse between inoculations of the central ribes to permit proper segregation of the resultant cankers on the pines. Plans for further tests on these plots were canceled as soon as it became possible to initiate similar studies in Idaho, and all central ribes were then destroyed.

On each selected area a study plot was established on which all pines within 150 feet (farther for the 1928 infection on two plots) of the central ribes were assigned a number and their locations carefully mapped. Crown length, crown width, and number of needles (*4*) were then determined for each of the trees for the same years in which the central ribes were inoculated. All trees were given several examinations at later dates to locate the cankers which had developed therein as a result of the infection on the central ribes.

While the danger of increasing the spread of the rust in the locality as a result of these experiments was practically nil, such remote danger as may have been involved was eliminated by selecting experimental areas within the infected regions of British Columbia where pine and ribes infection occurred sporadically and where, because of the scattered occurrence of the pine stocking and the high cost of control measures on these rugged sites, control measures were not contemplated.[12] Permission to conduct the experiments in the areas selected was granted by the proper Dominion and Provincial authorities prior to the establishment of the study plots.

CLIMATIC AND ECOLOGICAL CONDITIONS

In the selection of study areas in British Columbia the principal consideration was to locate them so as to approximate the climatic conditions found within the commercial range of western white pine in Idaho. In 1928 seven suitable areas were located, two approximately 40 miles and five approximately 100 miles north of the Idaho line, in the interior white pine belt of British Columbia, the northern extension of the white pine section of northern Idaho, western Montana, and northeastern Washington. The first two areas were near Nelson, at Hall and Apex, respectively, and the last five were near the north end of Slocan Lake at Hunter's Siding, near New Denver. The pertinent climatic data on record for these two localities in British Columbia are compared in table 1 with similar data for stations typical of the range of white pine in Idaho.

[12] DAVIDSON, A. T. RESULTS OF PAST SEASON'S WORK, PRESENT SITUATION, AND FUTURE PLANS. Western White Pine Blister Rust Conf., meeting of trustees, Spokane, Wash., 1924, Rept. Proc., pp. 7-20. [Mimeographed.]

——— BLISTER RUST ACTIVITIES IN BRITISH COLUMBIA. Western White Pine Blister Rust Conf., meeting of trustees, Seattle, Wash., 1924, Rept. Proc., pp. 9-11. [Mimeographed.]

TABLE 1.—*Comparison of general climatic records of stations near experimental plots in British Columbia with records from stations typical of the main commercial white pine section of Idaho*

Month	Precipitation [1]						Relative humidity [1]			Temperature [1]			
	British Columbia		Idaho				British Columbia	Idaho		British Columbia	Idaho		
	New Denver [2] (16 years)	Nelson (29 years)	Priest River (22 years)	St. Maries (27 years)	Avery (18 years)	Musselshell (9 years)	Nelson (11 years)	Priest River (7 years)	Avery (7 years)	Nelson (28 years)	Priest River (22 years)	St. Maries (27 years)	Avery (18 years)
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*	*Percent*	*°F.*	*°F.*	*°F.*	*°F.*
Jan.	3.44	3.14	3.67	3.27	4.19	5.22	88	.....	.....	24	23	28	27
Feb.	2.52	2.11	2.91	2.37	3.19	3.36	85	.....	.....	28	27	32	32
Mar.	2.37	1.82	2.46	2.64	3.71	4.68	74	.....	.....	37	35	39	38
Apr.	1.78	1.53	2.01	1.66	2.48	3.41	64	.....	.....	46	43	47	46
May	2.36	2.23	2.06	2.07	2.29	3.45	61	.....	.....	55	51	54	54
June	2.48	2.61	1.74	1.44	1.90	1.98	67	57	62	60	58	61	60
July	1.08	1.51	.82	.77	.91	1.21	59	46	56	66	64	67	67
Aug.	1.66	1.47	1.17	.82	1.04	.70	64	50	56	64	63	65	66
Sept.	1.80	1.85	1.77	1.27	1.68	1.83	74	63	61	56	53	57	58
Oct.	2.67	2.30	2.42	2.00	2.60	2.75	84	.....	.....	47	43	48	48
Nov.	2.73	2.91	3.72	3.63	3.97	4.47	88	.....	.....	35	33	38	36
Dec.	3.77	3.23	3.94	3.50	3.77	4.64	89	.....	.....	28	25	30	27

[1] Recorded average.
[2] Rain station only. Latest data for New Denver and Nelson, 1930. Latest data for Priest River Experiment Station, St. Maries, and Avery, 1933.

All study areas were located in old burns. The stands were typical of reproduction on burned areas in the white pine region, on which *Salix* spp., *Betula* spp. and *Populus tremuloides* Michx, are the most common deciduous species and western white pine the most abundant conifer. The vegetation had reached that successional stage wherein the conifers were just beginning to overtop and suppress the broadleaf species, except on two of the Hunter's Siding areas. The white pine reproduction on those two areas was much smaller, the burn being more recent and more severe than on the other areas.

The Hall and Apex plots had *Ribes viscosissimum* plants growing naturally at their centers, and three of the plots at Hunter's Siding had *R. lacustre* plants growing naturally at their centers. The two remaining plots at Hunter's Siding were free of ribes. *R. viscosissimum* bushes were transplanted at selected centers on these two latter plots.

The three plots at Hunter's Siding which had ribes growing naturally at the centers will hereafter be referred to as lacustre plot No. 1, lacustre plot No. 2, and lacustre plot No. 3. The two *Ribes viscosissimum* plots near Nelson will be designated as the Hall and Apex plots. The two plots at Hunter's Siding on which ribes plants were set out at the centers will be referred to as viscosissimum plot No. 1 and viscosissimum plot No. 2.

The Hall and Apex plots were natural *Ribes viscosissimum* sites. The soil consisted of well-drained clay loam, with a slight admixture of sand and gravel, and a thin humus layer. Both plots had medium slopes with southerly exposures. Their elevations approximated 3,000 feet. Vegetation was mixed broad-leaved and conifer, chief among the former being *Betula* spp., *Populus tremuloides*, *Alnus* spp., and *Salix* spp., and among the latter *Pinus monticola*, *P. contorta*

Dougl., *Pseudotsuga taxifolia* (Lam.) Britt., and *Larix occidentalis* Nutt. A fairly dense shrub story of *Ceanothus sanguineus* Pursh and *Pachistima myrsinites* Raf. was present on the Apex plot, and scattered bushes occurred on the Hall plot. White pine reproduction within the 150-foot radius on the Hall plot in 1928 ranged from 0.1 to 30.0 feet in height, mainly between 5 and 15 feet, and averaged 7.4 feet, and the stocking was equivalent to 156 pines per acre. Pines within the 150-foot radius on the Apex plot ranged from 0.1 to 34.5 feet high, averaging 7.8 feet, with 217 trees per acre in 1928.

The three *Ribes lacustre* plots at Hunter's Siding varied but little from 2,000 feet in elevation and were situated on gentle slopes with southerly exposures. Here again the sites were typical of the ribes species present. The tree species found were similar to those at Hall and Apex, with the addition of *Populus trichocarpa* Torr. and Gr. and *Acer glabrum* Torr. to the broad-leaved group and *Thuja plicata* D. Don and *Tsuga heterophylla* (Raf.) Sarg. to the coniferous group. The shrub cover consisted principally of *Rubus parviflorus* Nutt., *Pteridium aquilinum pubescens* Underw., *Cornus stolonifera* Michx., and *Pachistima*. The soil was similar on all plots, being a mixture of sand, gravel, and silt covered with a humus layer deeper than that on the Hall and Apex plots. Because of two small, multiforked creeks and numerous springs on lacustre plot No. 1 the amount of moisture on this plot was greater than on either of the two other *Ribes lacustre* plots. However, these streams were not in draws of sufficient depth to appreciably influence local air currents. There was a shallow draw through the center of lacustre plot No. 2 down which water flowed only in the very early spring. A small stream flowed through the center of lacustre plot No. 3 the entire year. This stream meandered through a flat, roughly 100 yards wide, adjacent to a hill arising on the western edge of the plot. In 1928 lacustre plot No. 1 supported 443 western white pines per acre, ranging from 0.3 to 30 feet high and averaging 10.4 feet high; lacustre plot No. 2 had 550 pines per acre, ranging from 0.1 to 43 feet high and averaging 5 feet high; and lacustre plot No. 3 had 164 pines per acre, ranging from 0.1 to 40 feet high and averaging 8.8 feet high.

The two *Ribes viscosissimum* plots at Hunter's Siding were on practically level ground, at an elevation of approximately 1,900 feet. The soil was a well-drained, deep, sandy loam, with a very thin layer of humus. Apparently these areas had been repeatedly burned until practically all organic matter in the top inch of soil had been destroyed. The vegetation was less dense but of the same composition as that found on the Hall and Apex plots. The shrubs *Ceanothus* and *Ribes* were absent. In 1930 the white pines on viscosissimum plot No. 1 ranged from 0.2 to 38 feet high and averaged 3.2 feet high, with 704 trees per acre. In 1930 on viscosissimum plot No. 2 the pines ranged from 0.3 to 35 feet high and averaged 3.9 feet high, with 359 trees per acre.

PROCEDURE

In the spring of 1928 the central ribes on the five plots that had ribes growing naturally at their centers were inoculated in the manner previously employed in the large-scale ribes-susceptibility studies (*9*), and in 1930 the ribes on all seven plots were inoculated. The central plants were periodically examined in the years in which they were not inoculated, as a check on the development of infection upon them.

From the time the plants were inoculated in the spring until their defoliation in the fall, in both 1928 and 1930, ribes examinations were made at approximately 3-week intervals, and the following types of data were recorded for the central plants: Feet of live stem, number of leaves, number of infected leaves, percentage of leaf surface infected on infected leaves, and percentage of infected surface bearing telia. The detailed method of recording ribes infection data has been described by Mielke et al. (*9*). A complete record of infection for each ribes group was thus secured.

Daily records of rainfall and relative humidity were secured from nearby Government weather stations for 1928 and 1930. In 1930 it was possible to have at least one man on the plots at nearly all times; so supplementary weather records were taken daily on each plot from the latter part of June until the ribes were defoliated in the fall. These records included duration of wet pine foliage, wind observations, humidity and temperature readings, and general sky and cloud conditions. Weather conditions undoubtedly exert a great influence on the development and spread of the rust (*10*), and some work has been published on the relation of moisture conditions to pine infection (*12*, *14*). The extreme complexity of this problem, however, makes impossible any accurate analysis of the correlation between pine infection and detailed environmental factors.

Two years after the 1928 ribes inoculations all pines on these plots were given a thorough examination for blister rust cankers. Periodic examinations were made thereafter until the late fall of 1933, when a total of seven examinations had been made. This allowed 3½ years after the 1930 ribes inoculation for all cankers to make an appearance. All cankers found were destroyed before they had produced aecia. The results of these examinations afforded some information on the distance of spread from the central ribes, and also, when correlated with volume of foliage exposed at varying distances, gave the intensity of infection. The following more detailed data were taken on each new canker found: Distance of canker or cankered branch from ground, side of tree, branch or stem, year's growth entered, and stage of development. All branch cankers were further segregated into one of the following categories: Will die before entering the bole; will enter the bole (years); or has entered the bole. Estimates were made on length of time required to kill or damage the tree for all cankers that were formed directly on the bole and for such branch cankers as were eventually to enter the bole, these estimates being based on knowledge of canker behavior and growth rates (*7*). Damage was considered to mean the death or malformation of any part of the tree sufficient to lower its ultimate merchantability. These data provided the necessary information for segregating cankers by year of origin (*6*), affording a measure of the infection and subsequent damage secured from each of the ribes inoculations.

Examination of pines on the plots at both Hunter's Siding and Nelson showed that appreciable infection from the 1928 ribes inoculations had spread beyond the limits of the original 150-foot circular plots. In 1930 the spread was again beyond the 150-foot boundaries of lacustre plots 1 and 3 at Hunter's Siding, but examination of the *Ribes viscosissimum* plots, both at Nelson and at Hunter's Siding, and of lacustre plot No. 2 showed that few if any cankers had originated beyond the 150-foot zone. At Hunter's Siding the areas on which the

R. lacustre plots were located were of such a character that to enlarge the plots would have involved a tremendous amount of labor and was considered impracticable because of the limited available personnel. The character of the areas near Nelson made the enlargement of the plots there a somewhat easier problem. To secure knowledge of maximum distance of spread, the two plots near Nelson, the Apex and Hall plots, were enlarged to a radius of first 200 feet and then 250 feet, as dictated by examination of pines for infection resulting from the 1928 inoculation of ribes. The Hall and Apex plots were not extended beyond the 150-foot radius for examination of pines for infection resulting from the 1930 inoculation.

A very few cankers were found on the areas near Nelson and Hunter's Siding which were clearly not the result of inoculations but of natural spread from associated ribes prior to their eradication from the areas. There is a remote possibility, therefore, that somewhere in the results there may be included a canker or two of doubtful origin, but such cankers are certainly not sufficiently numerous appreciably to alter the results of these studies.

RESULTS

Daily records of precipitation and relative humidity procured from the weather stations nearest to the study areas showed that in both 1928 and 1930 climatic conditions following inoculation of the central ribes were apparently favorable (*2, pp. 209–210; 12, 14*) for the development and uredial intensification of the rust on ribes, especially during the month of June. Conditions were not so favorable for pine infection during the period of telia development and production of sporidia, which normally occurs mainly from July (and even somewhat earlier on *Ribes lacustre*) to early October, precipitation in both years having been below the normal not only of the study localities but also of stations typical of the Washington-Idaho-Montana white pine region. Monthly precipitation records from the Nelson and New Denver weather stations and relative humidity records from Nelson are presented in table 2 as an index to the gross ribes- and pine-infection conditions that prevailed during each infection period.

TABLE 2.—*Comparison of moisture records during periods of possible rust development on ribes for 1928 and 1930, with long-term averages from weather stations nearest experimental plots in British Columbia*

Month	Precipitation [1]						Relative humidity [1]		
	Nelson			New Denver			Nelson		
	1928	1930	29 years	1928	1930	16 years	1928	1930	11 years
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*	*Percent*
April	2.53	1.65	1.53	1.58	1.40	1.78	60	67	64
May	2.50	3.50	2.23	1.69	3.40	2.36	53	65	61
June	4.03	3.56	2.61	4.30	2.56	2.48	68	70	67
July	1.17	.60	1.51	1.75	.78	1.08	65	60	59
August	.49	.68	1.47	.77	1.21	1.66	63	59	64
September	.42	.66	1.85	.25	1.14	1.80	63	68	74
October	3.14	3.15	2.30	4.67	3.54	2.67	79	84	84
November	1.89	1.99	2.91	2.53	.69	2.73	89	90	88

[1] Recorded average.

Table 3 shows the final ribes-infection data recorded for each test plot each year of inoculation, in comparison with the average as determined from previous large-scale ribes-susceptibility tests (*9*). With the exception of the Hall plot for 1930, all plots inoculated showed a higher percentage of leaf surface infected in both 1928 and 1930 than had been previously determined as the average. On all plots inoculated in 1928, the percentages of leaf surface bearing telia in that year were higher than average. In 1930 this figure was again higher than average for all *Ribes lacustre* plots and for the Apex plot, but was decidedly below average on the Hall plot and on the two *R. viscosissimum* plots at Hunter's Siding. The important information derived from table 3, however, is that telia were developed on all plots whenever the central ribes were inoculated, and the percentage of telia produced ranged from relatively high values on the Apex plot and on lacustre plot No. 2 in both years to a relatively low figure for viscosissimum plot No. 2 in 1930.

TABLE 3.—*Percentages of total leaf surface infected and bearing telia on ribes at centers of experimental plots in 1928 and 1930 in comparison with the previously determined average*

Plot	Ribes		Total leaf surface infected			Total leaf surface bearing telia		
	Species	Growth form [1]	1928	1930	Average [2]	1928	1930	Average [2]
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Lacustre No. 1	*R. lacustre*	Part shade	13.2	6.1	4.3	1.8	2.0	0.7
Lacustre No. 2	do	Open	29.4	26.5	3.4	7.5	8.7	.4
Lacustre No. 3	do	Part shade	6.6	8.0	4.3	1.0	5.7	.7
Apex	*R. viscosissimum*	Open	49.9	21.8	6.6	16.4	10.7	3.5
Hall	do	do	11.4	4.2	6.6	4.7	1.7	3.5
Viscosissimum No. 1	do	do	----	10.2	6.6	----	1.1	3.5
Viscosissimum No. 2	do	do	----	9.2	6.6	----	.6	3.5

[1] "Part shade"=growing in partial shade; "open"=growing in the open, fully exposed to the sun for most of the day.
[2] Averages from previous large-scale susceptibility tests (*10*).

Data secured on the factors of moisture and ribes infection as they existed on or near the study areas are graphically presented on a daily basis in figures 1 to 6.

The centers of viscosissimum plots Nos. 1 and 2 were but 18 chains apart on an extensive flat. For this reason it was not considered necessary to take separate weather records for each. Figure 6 shows the moisture data on these two plots for 1930, the only year in which their central ribes were inoculated.

In the absence of detailed records, morning dews were arbitrarily assumed (figs. 1–6) to have wet the pine foliage at 4 a. m.

The infections that occurred on the pines of each plot, under the conditions shown in figures 1 to 6, are summarized in table 4, along with the pertinent data on the ribes directly responsible for this pine infection. From this record it is fairly evident that conditions obtaining on all plots (figs. 1–6), with the exception of the Hall plot for 1930, were such as to enable considerable infection to spread from both *Ribes lacustre* and *R. viscosissimum* to the surrounding white pines. Analysis of weather records and ribes-infection data heretofore

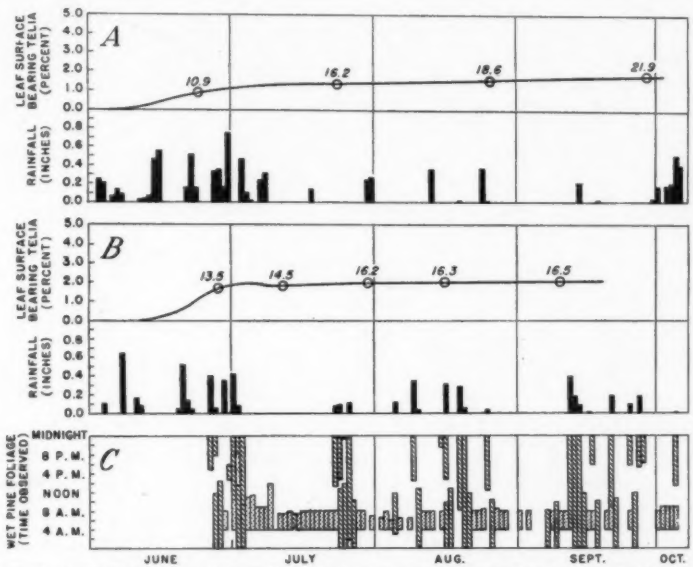

FIGURE 1.—Indices of pine-infection conditions during tests on lacustre plot No. 1, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). Data on telium production were taken only at the times indicated by the points on the curves. The form of curves between points was determined by estimates of the probable development of the rust between these times, based on the stage of development shown, on weather data, and on general knowledge of typical reaction to the rust of the ribes species concerned. The numbers at the points along the curves give the aggregate square inches of leaf surface that had borne telia up to the time indicated. The rainfall data were taken from records of the nearest weather station, New Denver. *C*, Periods of wet pine foliage, as observed on the plot from late June to early October 1930. Diagonal cross hatching represents wetting by rains; stippling, wetting by dews.

presented showed that the pine infection that resulted took place under very ordinary climatic conditions, such as might be expected to occur during almost any summer.

TABLE 4.—*Basis of ribes and pines and infection on each resulting from the 1928 and 1930 inoculations on all study plots*

Plot [1]	Inoculation year	Ribes				Pines				
		Growth form	Bushes	Live stem	Surface bearing telia [2]	Total trees	Average height	Trees infected		Total cankers [3]
			Number	*Feet*	*Square inches*	*Number*	*Feet*	*Number*	*Percent*	*Number*
Lacustre No. 1.	1928	Part shade.	10	62.4	21.9	719	10.4	79	11.0	127
	1930	....do....	10	74.2	16.5	715	12.9	52	7.3	101
Lacustre No. 2.	1928	Open.....	5	14.4	29.8	891	5.0	32	3.6	50
	1930	....do....	[4] 10	24.1	28.4	854	6.5	18	2.1	26
Lacustre No. 3.	1928	Part shade.	5	328.5	175.0	266	8.8	22	8.3	28
	1930	....do....	5	431.0	1,658.2	265	10.5	45	17.0	107
Apex (*R. viscosissimum*).	1928 [1]	Open.....	4	31.0	170.4	658	10.6	54	8.2	283
	1930	....do....	4	40.0	95.1	334	9.7	5	1.5	5
Hall (*R. viscosissimum*).	1928 [1]	....do....	5	141.0	181.0	776	8.0	28	3.6	43
	1930	....do....	5	179.0	69.4	251	9.3	0	0	0
Viscosissimum No. 1.	1930	....do....	20	59.5	24.5	1,141	3.2	21	1.8	25
Viscosissimum No. 2.	1930	....do....	21	80.3	7.3	581	3.9	16	2.8	18

[1] All plots were circular, with a radius of 150 feet, except Hall and Apex, which were extended to 250 feet in radius to include pine infections resulting from the 1928 ribes inoculation.

[2] Calculated by multiplying estimated percentage of leaf surface that bore telia by total leaf area. Total leaf area was calculated from measurements of a portion of the leaves from the central ribes at each test.

[3] The final pine examination was in the fall of 1933, thus allowing ample time for all cankers resulting from both inoculations to make their appearance.

[4] 5 additional bushes were transplanted at the center in 1929 to increase the footage of live stem.

To show variations in pine infection with increasing distance from the central ribes, data for all plots were summarized by concentric circles, each of which had a radius 50 feet greater than the preceding circle. An additional circular division was made at a radius of 117.75 feet to show results on the 1 acre closest to the central ribes. In obtaining these data it was found convenient to map the location of all pines and to show on the same chart the location of all infected trees with their respective number of cankers. For purposes of

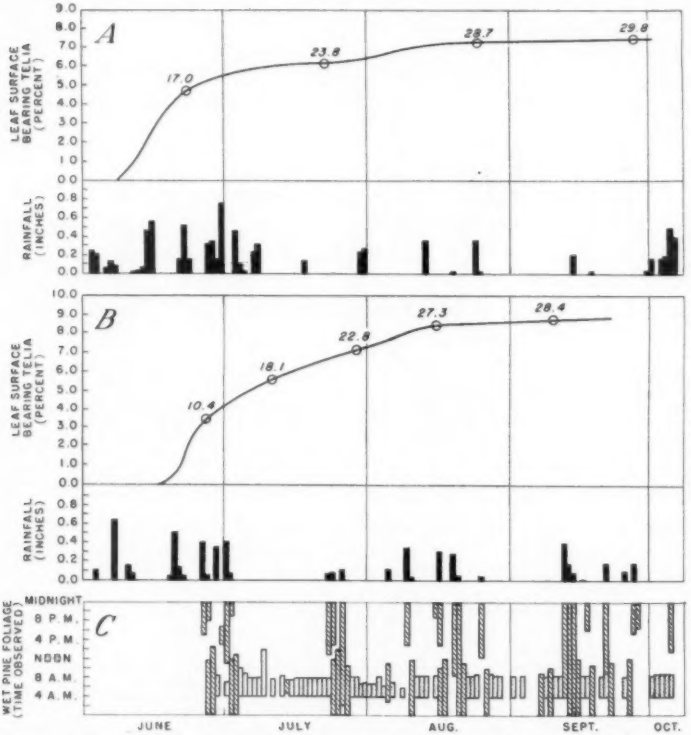

FIGURE 2.—Indices of pine-infection conditions during tests on lacustre plot No. 2, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from late June to early October 1930. (For further explanation of details, see legend of fig. 1.)

illustration figures 7 and 8 are given, showing the distribution of cankers resulting from ribes infection on lacustre plot No. 2 for 1930 and on the Apex plot for 1928. Distinctive spread patterns were found in only a few cases, the spread ordinarily assuming comparable proportions in all directions from the central ribes. With lacustre plot No. 2, however, a very definite distribution pattern was obtained in both 1928 and 1930. On this plot the central ribes were located in the bottom of a shallow draw, and a preponderance of cankers was formed along this draw, principally below the ribes, as shown in figure 7. The Hall and Apex plots both had definite slopes and here

again a skewed distribution pattern was secured, there being a strong tendency for the cankers to form on trees down the slope below the source of sporidia, as shown in figure 8 for the Apex plot.

Table 5 summarizes the pine infection and probable resultant damage for all three *Ribes lacustre* plots from both the 1928 and 1930 ribes inoculations. Table 6 similarly summarizes all pine infection and probable damage from the inoculation of *R. viscosissimum* test plots. In table 7 pine infection and probable damage within the circular acre are summarized for each plot individually for each year

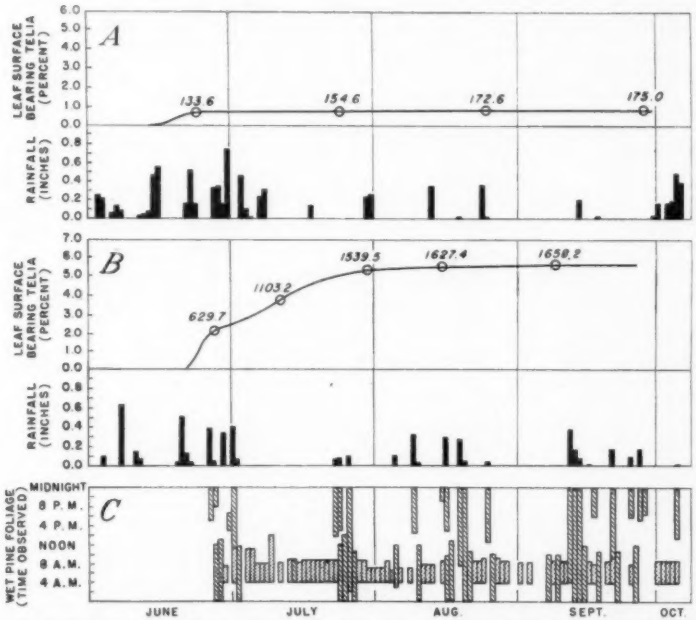

FIGURE 3.—Indices of pine-infection conditions during tests on lacustre plot No. 3, Hunter's Siding, near New Denver, British Columbia. Rainfall and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from late June to early October 1930. (For further explanation of details, see legend of fig. 1.)

of ribes inoculation. To complete the picture, the footage of ribes live stem and square inches of leaf surface bearing telia on each plot are included along with the pine-infection data. The individual plots showed wide variation in pine infection, which could not be explained solely upon the basis of probable sporidia production and concurrent moisture conditions. Pine infection is the result of many factors, and to accurately evaluate and correlate all of them would be highly impracticable if not impossible.

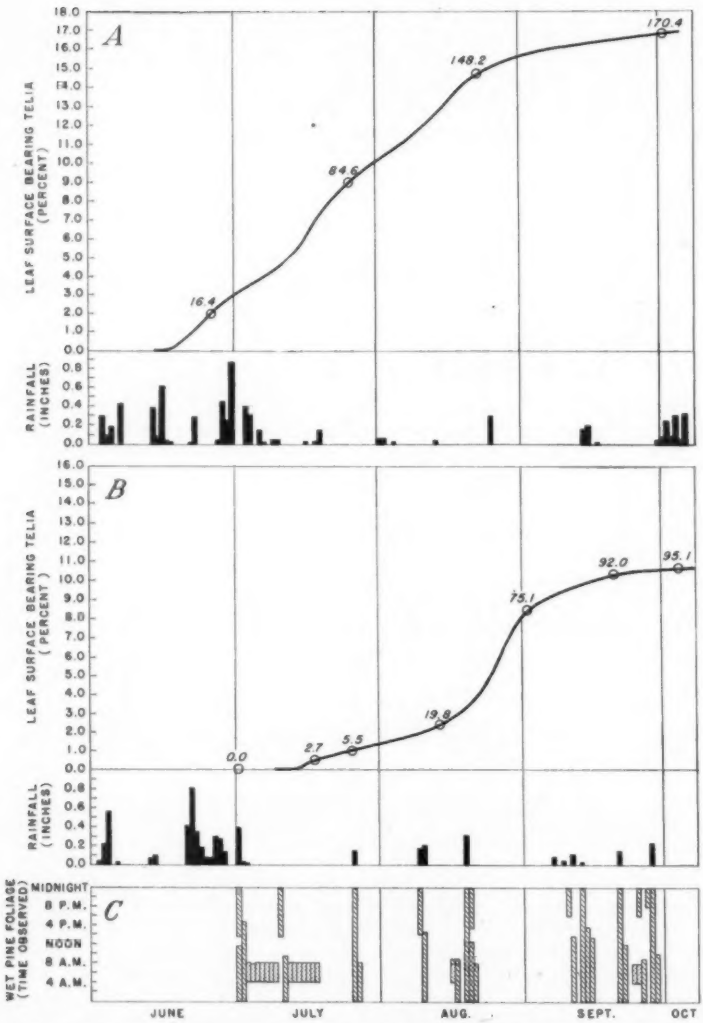

FIGURE 4.—Indices of pine-infection conditions during tests on Apex plot (*Ribes viscosissimum*), near Nelson, British Columbia. Rainfall (records from nearest weather station, Nelson) and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from July 1 through September 1930. (For further explanation of details, see legend of fig. 1.)

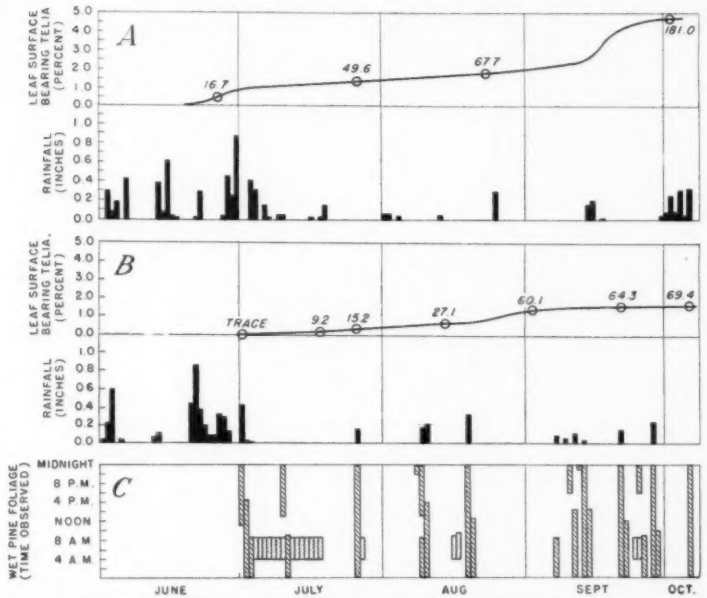

FIGURE 5.—Indices of pine-infection conditions during tests on Hall plot (*Ribes viscosissimum*), near Nelson, British Columbia. Rainfall (records from nearest weather station, Nelson) and percentage of total leaf surface that was bearing or had borne telia at any particular time (*A*, 1928; *B*, 1930). *C*, Periods of wet pine foliage as observed on the plot from July 1 to early October 1930. (For further explanation of details see legend of fig. 1.)

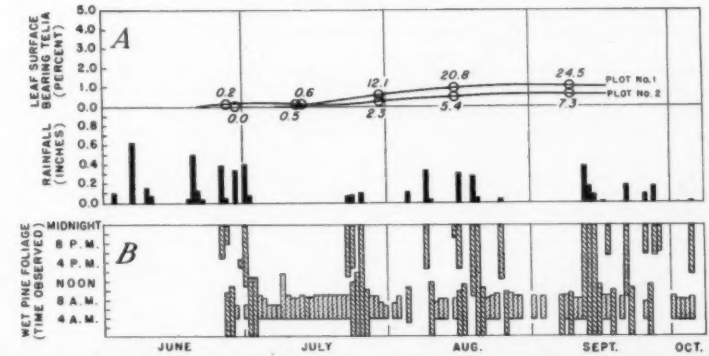

FIGURE 6.—Indices of pine-infection conditions during tests on viscosissimum plots No. 1 and No. 2, Hunter's Siding, near New Denver, British Columbia, 1930. *A*, (upper), Percentage of total leaf surface that was bearing or had borne telia at any particular time; (lower), rainfall data from records of nearest weather station, New Denver; *B*, periods of wet pine foliage as observed on the plots from late June to early October. (For further explanation of details, see legend of fig. 1.)

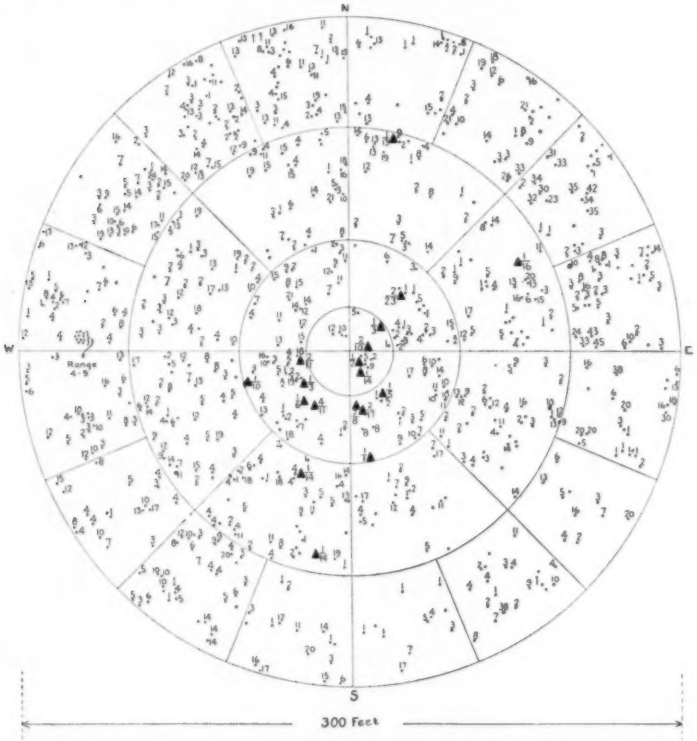

FIGURE 7.—Distribution of cankers resulting from infection in 1930 on *Ribes lacustre*, on lacustre plot No. 2, Hunter's Siding, near New Denver. Dots represent uninfected trees; tree heights of 1 foot or more are shown by numbers immediately above or next to each dot; infected trees are shown by solid triangles, the fractions adjacent to which give number of cankers over tree height.

TABLE 5.—*Spread and intensity of infection and prospective damage* [1] *to pines resulting from sporidia produced on Ribes lacustre, based on 6 tests on 3 plots in 1928 and 1930 at Hunter's Siding, British Columbia*

Distance from central ribes (feet)	Trees							Cankers			
	Total	Average height	Total infected		Total that will be damaged [2]		Infected trees that will be damaged [2]	Total [3]	Damage cankers [4]		Average time required to damage [1]
	Number	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Years*
0–50	476	7.8	68	14.3	31	6.5	45.6	158	42	26.6	10
50.1–100	1,412	9.1	100	7.1	32	2.3	32.0	139	34	24.5	11
100.1–150	1,821	8.3	80	4.4	36	2.0	45.0	142	42	29.6	12
0–100	1,888	8.8	168	8.9	63	3.3	37.5	297	76	25.6	11
0–117.75 [5]	2,753	8.9	206	7.5	80	2.9	38.8	346	94	27.2	11
0–150	3,709	8.6	248	6.7	99	2.7	39.9	439	118	26.9	11

[1] Damage = killing or injury sufficient to render the trees worthless for timber or timber production.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.
[4] Cankers which would have caused damage if they had not been cut out.
[5] 1 circular acre.

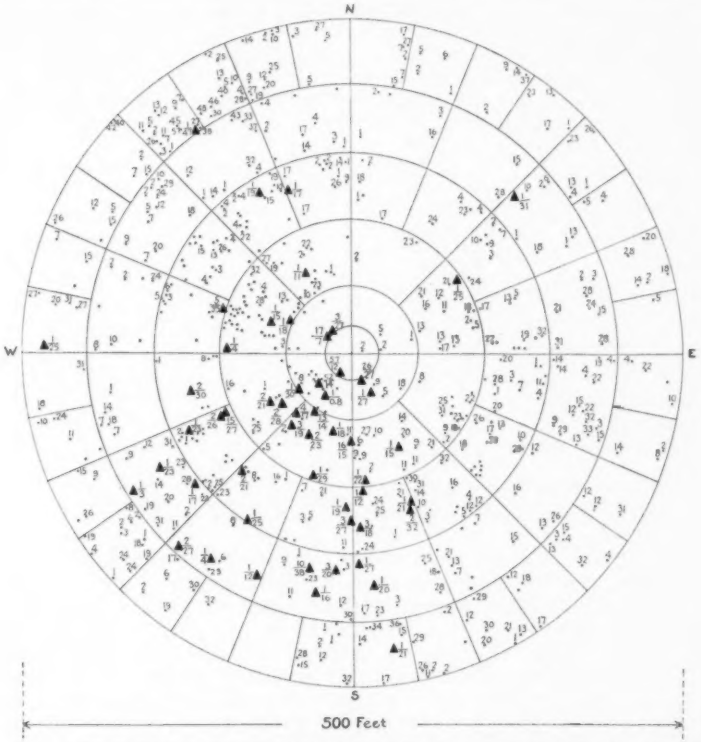

FIGURE 8.—Distribution of cankers resulting from infection in 1928 on *Ribes viscosissimum*, on Apex plot, near Nelson. Dots represent uninfected trees; tree heights of 1 foot or more are shown by numbers immediately above or next to each dot; infected trees are shown by solid triangles, the fractions adjacent to which give number of cankers over tree height.

TABLE 6.—*Spread and intensity of infection and prospective damage* [1] *to pines resulting from sporidia produced on Ribes viscosissimum, based on 4 tests on 2 plots in 1928 and 1930 near Nelson and 2 tests on 2 plots in 1930 at Hunter's Siding, British Columbia*

Distance from central ribes (feet)	Trees							Cankers			
	Total	Average height	Total infected		Total that will be damaged [2]		Infected trees that will be damaged [2]	Total [3]	Damage cankers [4]		Average time required to damage [1]
	Number	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Number*	*Percent*	*Years*
0–50	345	4.9	42	12.1	24	6.9	57.1	220	34	15.4	9
50.1–100	1,006	5.8	29	2.8	12	1.1	41.3	64	16	25.0	11
100.1–150	1,560	5.4	32	2.0	9	.6	28.1	57	10	17.5	12
150.1–200 [5]	341	10.1	17	5.0	6	1.8	35.3	29	7	24.1	14
200.1–250 [5]	489	10.6	4	.8	1	.2	25.0	4	1	25.0	21
0–100	1,351	5.5	71	5.2	36	2.6	50.7	284	50	17.6	10
0–117.75 [6]	1,832	5.8	83	4.5	43	2.3	51.8	315	58	18.4	10
0–150	2,911	5.5	103	3.5	45	1.5	43.6	341	60	17.5	10
0–200 [5]	3,252	6.0	120	3.7	51	1.6	42.5	370	67	18.1	11
0–250	3,741	6.6	124	3.3	52	1.4	41.9	374	68	18.2	11

[1] Damage=killing or injury sufficient to render the trees worthless for timber or timber production.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.
[4] Cankers which would have caused damage if they had not been cut out.
[5] The Hall and Apex plots were extended to a 250-foot radius to trace out the pine infection for 1928. The other four tests included in this table extended 150 feet from the central ribes.
[6] 1 circular acre.

TABLE 7.—*Pine infection and subsequent damage within 1 circular acre surrounding a central group of ribes, resulting from a single inoculation of the ribes on each of the 7 test plots*

Plot	Inoculation year	Ribes		Pines on the central circular acre [1]						Total cankers [3]
		Live stem	Leaf surface bearing telia	Total	Average height	Infected		Damaged [2]		
		Feet	*Square inches*	*Number*	*Feet*	*Number*	*Percent*	*Number*	*Percent*	*Number*
Lacustre No. 1	1928	62.4	21.9	557	10.5	64	11.5	32	5.8	109
	1930	74.2	16.5	555	13.4	39	7.0	9	1.6	45
Lacustre No. 2	1928	14.4	29.8	618	5.6	30	4.9	17	2.8	47
	1930	24.1	28.4	567	6.6	18	3.2	5	.9	26
Lacustre No. 3	1928	328.5	175.0	230	8.1	16	7.0	3	1.3	22
	1930	431.0	1,658.2	226	9.8	39	17.3	14	6.2	97
Apex (*R. viscosissimum*)	1928	31.0	170.4	232	8.9	29	12.5	13	5.6	239
	1930	40.0	95.1	194	10.7	3	1.5	2	1.0	3
Hall (*R. viscosissimum*)	1928	141.0	181.0	161	7.7	18	11.2	6	3.7	33
	1930	179.0	69.4	159	9.3	0	.0	0	.0	0
Viscosissimum No. 1	1930	59.5	24.5	707	3.1	18	2.6	16	2.3	23
Viscosissimum No. 2	1930	80.3	7.3	379	4.0	15	4.0	6	1.6	17

[1] 1 circular acre has a radius of 117.75 feet.
[2] Trees that would have been damaged if cankers formed thereon had not been cut out.
[3] Includes both damage cankers and those that would have died without causing serious injury.

The number of cankers formed at increasing distances from the central ribes, as averaged for all tests, is shown by the curve in figure 9, in which number of cankers per million needles is plotted over distance from plot center in feet. A measure of the intensity of pine infection at varying distances from the plot center was also secured by plotting percentage of total cankers within a given zone, divided by percentage of total needles within that same zone, over distance from plot center in feet. These data are graphically presented in figure 10 for each plot for each year of inoculation. These curves show that in all cases the intensity of infection fell to almost negligible values between 50 and 60 feet from the central ribes irrespective of how great the intensity may have been closer to the ribes.

In analyzing these curves it should be borne in mind that, although there is a decided reduction in intensity of infection beyond 50 or 60 feet from the central ribes, there is still a considerable number of cankers formed at even greater distances. Trees close to the central ribes are very likely to receive new damaging infections with each successive year of ribes infection—a duplication of damage that is not particularly significant, because one damage canker alone is sufficient to cause the loss of a tree. Duplication of damage is less likely to occur on the more distant trees, as the sporidia will be more and more widely dispersed with increasing distance from the ribes. For example, table 5 shows that 31 trees were damaged in the 0- to 50-foot circles, 32 were damaged in the 50.1- to 100-foot circles, and 36 were damaged in the 100.1- to 150-foot circles. It is therefore apparent that, while percentage of damage may decrease rapidly beyond the 150-foot circle, the total damage beyond this point may exceed that within 150 feet of the ribes.

DISCUSSION

The artificial inoculation exposed the central ribes on the test plots to a greater volume of aeciospores and resulted in heavier infection and subsequent telia production than would occur in nature unless the ribes were exposed to a nearby and abundant source of aecia. Such

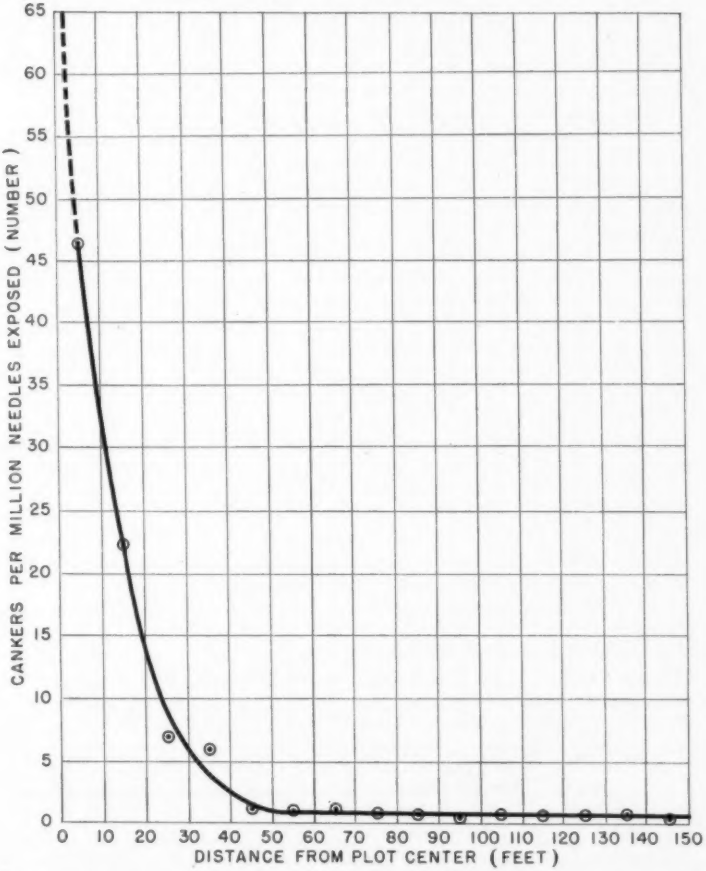

FIGURE 9.—Average number of cankers per million needles exposed in 10-foot zones out from the central ribes, for all plots combined.

association would be only occasional in the first stages of natural invasion of the rust into a previously rust-free area. In older centers of pine infection such associations might readily occur with alarming frequency. The time required for such an aecial supply to be built up in a previously rust-free area is extremely variable and depends to a considerable extent on the original intensity of ribes infection, the ribes-pine association, and the climatic conditions that prevailed

during these developmental years. In view of the great importance of the stage of development of ribes leaves at the time of inoculation to their susceptibility, as shown by Lachmund (*8*), it is possible that natural inoculations, where ribes are growing near fruiting cankers, might in some cases result in greater development of the rust and

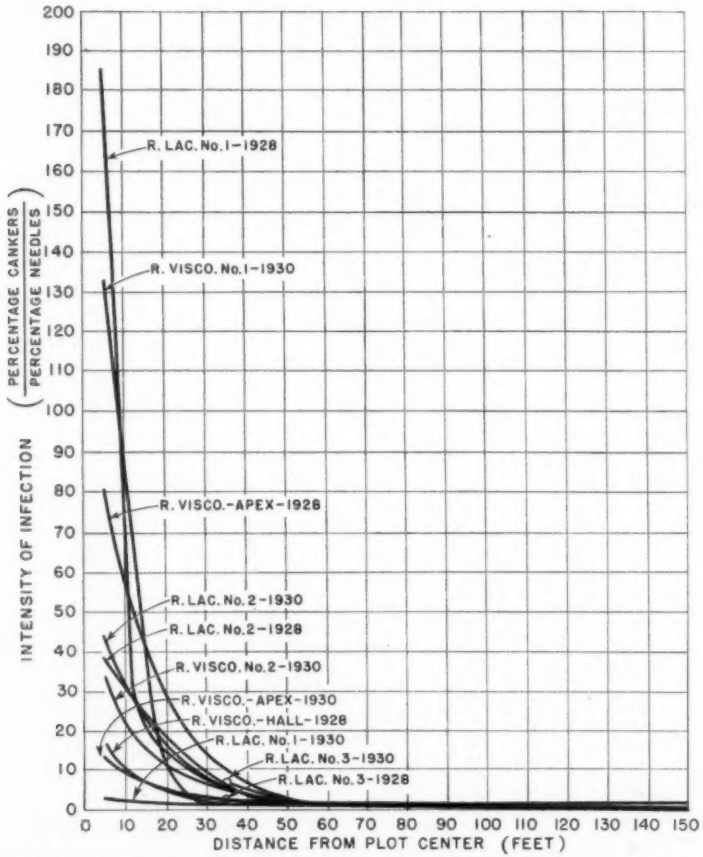
INTENSITY OF INFECTION (PERCENTAGE CANKERS / PERCENTAGE NEEDLES)
DISTANCE FROM PLOT CENTER (FEET)
R. LAC. No. 1-1928
R. VISCO. No. 1-1930
R. VISCO.-APEX-1928
R. LAC. No. 2-1930
R. LAC. No. 2-1928
R. VISCO. No. 2-1930
R. VISCO.-APEX-1930
R. VISCO.-HALL-1928
R. LAC. No. 1-1930
R. LAC. No. 3-1930
R. LAC. No. 3-1928
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200

FIGURE 10.—Reduction of pine-infection intensity with increasing distance from ribes at plot centers. The measure of infection intensity consists of the percentage of cankers divided by the percentage of needles in each 10-foot concentric zone out from the center on each plot. Smoothed curves were drawn through the points so derived for each plot. The points are not shown for lack of space but in general fall on or close to their respective curves.

greater damage to pines than from artificial inoculations, even though the latter are purposely made very heavy. It is conceivable, then, for ribes in any white pine stand to be exposed eventually to quantities of aecia sufficient to cause ribes infection and subsequent telia production comparable to that secured on the test plots as shown in table 3.

Under the conditions of ribes infection obtaining in the tests, damaging pine infection resulted from groups of ribes having from 431.0 feet of live stem to as little as 14.4 feet of live stem, which was the smallest amount present on any plot. Such a multiplicity of factors was involved, however, that it was impossible to directly correlate number of feet of ribes live stem with resultant pine infection. Lacustre plot No. 3, with 431.0 feet of *Ribes lacustre* in 1930, had the greatest footage of live stem of any plot. On this plot 17.0 percent of the pines became infected, the highest percentage shown on any plot. For the Hall plot in 1930, directly opposite results were secured, 179.0 feet of *R. viscosissimum* live stem causing not a single canker. The inconsistency is further shown by the results for lacustre plot No. 2 for 1928, on which but 14.4 feet of live stem infected 3.6 percent of the pines, and by the Apex plot in 1928, where but 31.0 feet of *R. viscosissimum* infected 8.1 percent of the pines. The results of these studies, therefore, do not justify the conclusion that a definite footage of live stem of either *R. lacustre* or *R. viscosissimum* will necessarily be responsible for a definite amount of pine infection. The results do justify the conclusion that even a few feet of live stem may and probably will cause appreciable pine infection under conditions comparable to those prevailing on the study areas in British Columbia.

Combining the results from all *Ribes lacustre* tests, it was found that 7.5 percent of the pines within the circular acre became infected from an average of 155.8 feet of ribes live stem in a single year. Similarly, on the *R. viscosissimum* plots 4.5 percent of the pines within the circular acre became infected from an average of 88.5 feet of ribes live stem. If the live stem on the *R. viscosissimum* plots were increased to equal that on the *R. lacustre* plots, then by simple proportion the *R. viscosissimum* plots would show 7.9 percent of the pines infected from that amount of live stem. On this basis equal footages of ribes live stem of the two species might be considered potentially capable of spreading approximately equal amounts of infection to the pines within a circular acre. From these tests it was determined that both ribes species were capable of spreading appreciable infection to pines for a distance of at least 150 feet; and, although the maximum distance of spread in these tests was not determined for *R. lacustre*, it was found that under the conditions existing on the plots *R. viscosissimum* was capable of spreading infection to pines for a distance of about 250 feet. In other studies considerably longer spread has been observed under other conditions, especially with other species of ribes (*11*).

It is pine damage, however, that is of practical importance, rather than ribes infection or pine infection as such. On every test plot and in every year in which pine infection took place as a final result of the inoculation of the central ribes, there occurred appreciable pine damage. As all plots were understocked, the loss of any tree meant actual loss in the board-foot potentialities of the stand. As might be anticipated by those familiar with the rust, percentage damage became less severe with increasing distance from the central ribes (tables 5 and 6). This reduction in damage was solely because of the reduction in intensity of infection with increasing distance from the ribes. The results of these studies showed that in general one out of every four cankers formed on the *Ribes lacustre* plots would probably cause damage, while the proportion was roughly 1 in 5 for the *R. viscosissimum* plots. There is no reason for considering cankers formed from

sporidia produced on *R. lacustre* to be more predisposed to cause damage than cankers initiated by sporidia borne by *R. viscosissimum*. That the ratio of damage cankers to total cankers was higher for plots having *R. lacustre* at their centers than for those with *R. viscosissimum* appears to be largely the result of differences in the character of the pine target exposed.

The ratios of pine damage to pine infection serve to emphasize the fact that under conditions as found on the study plots even relatively light pine infection will result in appreciable damage to the stand. In general, the results showed that this damage would occur within 10 to 15 years following infection of the pines. In these very young stands it does not appear important whether damage occurs in the first year following pine infection or not until the thirtieth year, the pines having no chance to reach merchantability in either case.

Table 5 shows that on the average for all *Ribes lacustre* plots tested 2.9 percent of all pines within the circular acre would suffer sufficient damage, from a single year's ribes infection, to prevent those trees from reaching merchantability. Table 6 shows that 2.3 percent of all trees within the circular acre on the *R. viscosissimum* plots would be similarly damaged. Under infection conditions found on the test plots it is readily apparent that a 15- to 25-percent reduction in stocking could easily occur within a relatively few years. A reduction even within these limits might mean the difference between profit and loss in the management of a young western white pine stand.

It has been shown that the heaviest pine infection and damage occur closest to the ribes. With an amount of ribes equal to that found at the centers of various test plots scattered, as the plants occur in nature, indiscriminately throughout the acre, it would be conservative to estimate damage as being only equal to that secured from a central group.[13] Since fall precipitation during the inoculation years was below average, it appears probable that pine-infection conditions were somewhat less favorable than would ordinarily be expected; this, together with the frequent spread of infection beyond the plot boundaries, indicates that the recorded infection is in no sense to be considered extreme for the given quantities of telia or live stem, but on the contrary must be considered conservative.

SUMMARY

For the purpose of securing more definite data on the capacity of the prickly currant (*Ribes lacustre*) and of the sticky currant (*R. viscosissimum*) to spread damaging white-pine blister rust (*Cronartium ribicola*) infection to western white pine (*Pinus monticola*), so-called ribes-to-pine spread studies were initiated in 1928 in British Columbia, where climatic conditions were reasonably comparable to those in northern Idaho. In these studies, infection was permitted to spread from central groups of artificially inoculated ribes of known footages of live stem to the surrounding stand of otherwise ribes-free white pine reproduction.

Detailed records of telia produced on the central ribes, the pine target exposed to sporidia developed from these telia, and the number and location of cankers formed provided the necessary data for computing the distance and intensity of spread within the plot boundaries

[13] Mielke [18] has shown how *Ribes lacustre*, scattered naturally throughout a stand, will intensify infection in the pines.

and the resultant damage to pines from each of the two ribes species tested. Climatic data were recorded during test years.

Analysis of these data indicated that under the test conditions equal footages of live stem of the sticky currant and prickly currant are capable of spreading essentially equal infection to nearby white pine reproduction, although no direct and consistent relationship between feet of ribes live stem and resultant pine infection was apparent for either ribes species tested. Within a circular acre, averages from the six tests of *Ribes lacustre* showed that from infection supported by 155.8 feet of live stem 7.5 percent of the pines became infected in a single year. With six tests of *R. viscosissimum*, an average of 88.5 feet of live stem caused infection on 4.5 percent of the pines within a circular acre.

The results further showed that both ribes species were capable of spreading appreciable infection to pines for a distance of at least 150 feet. Under the test conditions *Ribes viscosissimum* spread some infection for a distance of about 250 feet. For both species the intensity of infection was heaviest near the ribes and fell off to relatively low values at a distance of 50 to 60 feet from the central ribes. Percentage of pine damage follows a similar curve of reduction with increasing distance from the ribes. From an average of six tests, 155.8 feet of *R. lacustre* live stem supported infection which spread damage to 2.9 percent of the pines within a circular acre. From an average of six tests, 88.5 feet of *R. viscosissimum* live stem supported infection which spread damage to 2.3 percent of the pines within a circular acre.

Under conditions comparable to those prevailing on the test plots during the years in which the central ribes were inoculated it is possible for as little as 14.4 feet of *Ribes lacustre* live stem to be responsible for damage to 2.8 percent of the white pine reproduction within a circular acre from a single season of ribes infection. Similarly, as little as 31.0 feet of *R. viscosissimum* live stem is potentially capable of supporting infection causing damage, within a single season, to 5.6 percent of the pines within a circular acre.

These studies are significant in indicating that both *Ribes lacustre* and *R. viscosissimum* must be removed from areas that are to be managed for the production of western white pine. Under some conditions, at least, the eradication must be extremely thorough. It is shown that less than 15 feet of ribes live stem can cause damage to stands of white pine reproduction if the ribes bushes are subject to heavy infection, a condition that will exist in areas where sporulating cankers are numerous on associated pines.

LITERATURE CITED

(1) ANONYMOUS.
1927. WHITE PINE BLISTER RUST, ITS CAUSE AND CONTROL IN THE WEST. Timberman 28: 56–61, illus.

(2) ARTHUR, J. C., in collaboration with KERN, F. D., ORTON, C. R., FROMME, F. D., JACKSON, H. S., MAINS, E. B., and BISBY, G. R.
1929. THE PLANT RUSTS (UREDINALES). 446 pp., illus. New York and London.

(3) BEDWELL, J. L.
1928. THE CONTROL OF WHITE PINE BLISTER RUST IN THE WEST. Univ. of Wash. Forestry Club Quart. 6 (2–4): 13–20, illus.

(4) BUCHANAN, T. S.
1936. AN ALINEMENT CHART FOR ESTIMATING NUMBER OF NEEDLES ON WESTERN WHITE PINE REPRODUCTION. Jour. Forestry 34: 588–593, illus.

(5) EASTHAM, J. W.
1922. WHITE-PINE BLISTER RUST IN B. C. . . . Agr. Jour. [Brit. Columbia] 7: 29, 41, 57, 64.
(6) LACHMUND, H. G.
1933. METHOD OF DETERMINING AGE OF BLISTER RUST INFECTION ON WESTERN WHITE PINE. Jour. Agr. Research 46: 675–693, illus.
(7) ———
1934. GROWTH AND INJURIOUS EFFECTS OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA. Jour. Agr. Research 48: 475–503, illus.
(8) ———
1934. SEASONAL DEVELOPMENT OF RIBES IN RELATION TO SPREAD OF CRONARTIUM RIBICOLA IN THE PACIFIC NORTHWEST. Jour. Agr. Research 49: 93–114, illus.
(8a) MIELKE, J. L.
1938. AN EXAMPLE OF THE ABILITY OF RIBES LACUSTRE TO INTENSIFY CRONARTIUM RIBICOLA ON PINUS MONTICOLA. Jour. Agr. Research 55: 873–892, illus.
(9) ——— CHILDS, T. W., and LACHMUND, H. G.
1937. SUSCEPTIBILITY TO CRONARTIUM RIBICOLA OF THE FOUR PRINCIPAL RIBES SPECIES FOUND WITHIN THE COMMERCIAL RANGE OF PINUS MONTICOLA. Jour. Agr. Research 55: 317–346, illus.
(10) PENNINGTON, L. H.
1925. RELATION OF WEATHER CONDITIONS TO THE SPREAD OF WHITE PINE BLISTER RUST IN THE PACIFIC NORTHWEST. Jour. Agr. Research 30: 593–607, illus.
(11) POSEY, G. B., and FORD, E. R.
1924. SURVEY OF BLISTER RUST INFECTION ON PINES AT KITTERY POINT, MAINE, AND THE EFFECT OF RIBES ERADICATION IN CONTROLLING THE DISEASE. Jour. Agr. Research 28: 1253–1258, illus.
(12) SPAULDING, P., and GRAVATT, A. R.
1925. CONDITIONS ANTECEDENT TO THE INFECTION OF WHITE PINES BY CRONARTIUM RIBICOLA IN THE NORTHEASTERN UNITED STATES. Phytopathology 15: 573–583, illus.
(13) WYCKOFF, S. N.
1930. BLISTER RUST CONTROL IN THE INLAND EMPIRE. Timberman 31: 162–168.
(14) YORK, H. H., and SNELL, W. H.
1922. EXPERIMENTS IN THE INFECTION OF PINUS STROBUS WITH CRONARTIUM RIBICOLA. Phytopathology 12: 148–150.

RELATION OF THE CURLY TOP VIRUS TO THE VECTOR, EUTETTIX TENELLUS [1]

By C. W. BENNETT, *pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry,* and HUGH E. WALLACE, *associate entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

Association of the beet leafhopper, *Eutettix tenellus* (Baker), with a disturbance in beet called "curly leaf" or "blight," now known to have been curly top, was discovered by Ball (*1*)[2] through extensive field observations in Utah. Following this pioneer work, a number of investigators contributed to the knowledge of the relation of the causal agent of the disease to the insect vector.

Shaw (*16*) demonstrated that the feeding of a single leafhopper for only a few minutes was sufficient to initiate curly top on small beets. Smith and Boncquet (*17*) found that a 5-minute feeding period was sufficient for single viruliferous leafhoppers to cause infection.

Boncquet and Hartung (*3*) found that leafhoppers taken from certain natural breeding areas contained no virus, and Stahl and Carsner (*18*) showed that each leafhopper must feed on a plant infected by the virus before it is able to transmit the disease. Severin (*10*) stated that lots of 25 to 50 leafhoppers were able to cause infection in 4 to 6 hours after first feeding on an infected plant. He (*11*) also reported that leafhoppers retained their ability to produce infection throughout their nymphal and adult life, although ability to produce infection decreased with age in the case of leafhoppers transferred daily on beets. Later, however, he (*13*) found that the virus was lost by overwintering females in an average time of 83.9 days.

Carsner and Stahl (*4*) showed that insects caged singly on healthy plants were able to cause infection in 22 hours 45 minutes after having previously fed on infected plants. In experiments carried out by Severin (*12*), individual leafhoppers were able to transmit virus 7 hours after first feeding on an infected plant, and lots of 100 or more were able, in a few instances, to transmit virus in 20 to 30 minutes after first feeding on an infected plant. Nonviruliferous leafhoppers picked up virus in a feeding period of 1 minute, and viruliferous leafhoppers infected healthy plants in a feeding period of 1 minute. Mouth parts were found contaminated with virus after the leafhoppers had fed on diseased plants 30 to 60 minutes. No virus was recovered from feces.

Since curly top transmission is produced consistently only through the agency of the beet leafhopper, a knowledge of the relations of the virus to its insect vector is of considerable importance in the study of other phases of the curly top problem. During the past 6 years, as a part of the curly top investigations conducted at Riverside, Calif., a number of tests have been made, at irregular intervals, to gain more information on this subject. The results of these tests are presented in this paper.

[1] Received for publication Apr. 17, 1937; issued March 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 50

RELATION OF PERIOD OF FEEDING TO ACQUISITION OF VIRUS

Tests were made to determine (1) the minimum time required for leafhoppers to pick up virus from diseased plants and (2) the influence of length of the feeding period on the percentage of fed leafhoppers that became viruliferous. For these tests, adult nonviruliferous leafhoppers were starved for several hours and then caged on diseased beet (*Beta vulgaris* L.) plants for different time intervals ranging from 1 minute to 24 hours. The leafhoppers that fed 1 to 10 minutes, inclusive, were caged singly on diseased beet leaves and watched under a hand lens until they started feeding. The time was noted and the leafhoppers were watched until the expiration of the allotted feeding period, when they were removed and caged on healthy seedling beets for 7 days. Leafhoppers that did not feed continuously during the allotted feeding period were discarded. In feeding periods greater than 10 minutes the leafhoppers were placed in groups of 15 to 20 on diseased plants and left undisturbed during the feeding period. They were then caged singly on seedling beets for 7 days. Leafhoppers reared on diseased plants were used as checks. The feedings on diseased plants were made at temperatures of 95° to 110° F. In this temperature range previously starved leafhoppers usually feed continuously for prolonged periods.

In this experiment leafhoppers picked up virus in a feeding time of 1 minute. The percentage of leafhoppers that acquired virus steadily increased with increase in feeding period up to 5 hours, after which no decided increase was evident up to and including the 24-hour period. However, the check leafhoppers, which were reared to the adult stage on curly top beets, produced more infection than any of the other groups. A summary of the results of these tests is presented in table 1.

TABLE 1.—*Relation of period of feeding of nonviruliferous leafhoppers on curly top plants to acquisition of ability to infect seedling beets*

[150 plants inoculated in each test]

Feeding period (minutes)	Plants infected		Feeding period (hours)	Plants infected	
	Number	*Percent*		*Number*	*Percent*
1	5	3.3	1	66	44.0
2	10	6.7	2	81	54.0
3	13	8.7	3	90	60.0
4	18	12.0	4	93	62.0
5	22	14.7	5	115	76.7
10	23	15.3	6	113	75.3
20	40	26.7	12	110	73.3
30	42	28.0	24	114	76.0
			Check [1]	129	86.0

[1] Leafhoppers reared on curly top plants.

Further tests were made in an attempt to determine the rate at which leafhoppers pick up virus and the feeding time required to obtain a maximum charge. In preparation for this experiment a large number of female nonviruliferous leafhoppers were placed on a healthy beet plant. After 2 days these leafhoppers were removed and the eggs that had been deposited were allowed to hatch. While the nymphs were quite small they were divided into two approximately equal lots. One lot remained on a healthy plant and the other lot was placed on a curly top plant. As soon as all the leafhoppers had

reached the adult stage the nonviruliferous lot was placed on a curly top plant and tested for ability to infect seedling beets and for total virus content at intervals for 3 weeks. Tests were made also of the viruliferous lot, which served as a check on the rate of acquisition of virus by the previously nonviruliferous lot. The two kinds of tests were made as follows: (1) Twenty leafhoppers from each lot were caged singly on seedling beets for 24 hours. The number of plants infected was taken as a relative measure of the ability of the two lots of leafhoppers to transmit virus. (2) The leafhoppers, removed from the seedling beets after 24 hours, were macerated in distilled water and centrifuged, and the supernatant liquid was added to an equal volume of 95-percent alcohol. The resulting precipitate was thrown down by centrifugation, dried, suspended in 1 cc of 5-percent sugar solution, and centrifuged, and the supernatant liquid was reserved for testing. Twenty nonviruliferous leafhoppers were allowed to feed on each test liquid 6 hours, after which they were caged singly on seedling beets 7 days. The number of plants infected was considered a relative measure of the virus content of the two lots of leafhoppers.

The results of this experiment (table 2) indicate that the ability of the test leafhoppers to transmit virus increased rapidly and reached a maximum in 2 to 6 days. During the remainder of the test it was reasonably uniform and approximately equal to that of the viruliferous checks. In contrast to this the virus content of the test leafhoppers increased at a relatively slow rate and reached its maximum on the fourteenth day. Under the conditions of this experiment, the virus content required to enable a leafhopper to reach its maximum efficiency as a vector was considerably less than the maximum charge it is capable of carrying.

TABLE 2.—*Rate of increase in transmissive ability and in virus content of previously nonviruliferous leafhoppers when placed on curly top plants*

Inoculations by means of—	Group of leafhoppers tested [1]	Infection [2] from placing of nonviruliferous leafhoppers on curly top plants for—									
		0 [3] day	1 day	2 days	3 days	4 days	6 days	8 days	10 days	14 days	21 days
		Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Direct feeding, 24 hours.	Originally nonviruliferous.	0.0	32.5	60.0	55.0	67.5	70.0	57.5	62.5	70.0	62.5
	Check	70.0	80.0	62.5	87.5	72.5	70.0	67.5	55.0	52.5	47.5
Extracts from leafhoppers used in tests.	Originally nonviruliferous.	.0	5.0	17.5	15.0	32.5	30.0	22.5	35.0	50.0	45.0
	Check	50.0	47.5	50.0	55.0	50.0	47.5	57.5	72.5	55.0	47.5

[1] The "originally nonviruliferous" leafhoppers were nonviruliferous until placed on curly top beets at the beginning of the experiment; the "check" leafhoppers were reared on curly top plants and remained on curly top plants until tested.
[2] Calculated on the basis of 40 plants that were inoculated in each interval in each test.
[3] Tests made immediately before placing nonviruliferous colonies on curly top beets.

RELATION OF PERIOD OF FEEDING TO INFECTION

In tests of viruliferous leafhoppers to determine the relation of the period of feeding to infection, adult individuals were placed in large cages and starved for several hours. They were then allowed two short feeding periods on healthy beet plants to avoid the effect that starvation has on infection in the first feedings after the starvation

period (see section on effect of fasting period on infection, p. 36). They were then caged singly on cotyledons of seedling beets, watched under a hand lens, and their feeding period recorded. The feedings were made at a temperature of 90° to 110° F., and the feeding periods ranged from ½ to 30 minutes. The tests were repeated at intervals over a period of several months. The plants on which the leafhoppers fed were kept 1 month to determine the number infected.

The results of this series of tests, together with results from 4- and 6-minute tests compiled from other sources, are presented in table 3. No infection was produced in a feeding time of one-half minute and very little in 1 minute. The percentage of infection rose rapidly with increase in the feeding period up to and including the 3-minute feeding period, after which the rate of increase was less.

TABLE 3.—*Relation of period of feeding of viruliferous leafhoppers to infection of seedling beets*

Feeding period (minutes)	Plants inoculated	Plants infected		Feeding period (minutes)	Plants inoculated	Plants infected	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
½	52	0	0.0	6	3,376	1,200	35.5
1	72	1	1.4	10	140	48	34.3
2	343	23	6.7	11 to 15	83	40	48.2
3	159	35	22.0	16 to 20	65	34	52.3
4	87	19	21.8	21 to 25	51	28	54.9
5	567	150	26.5	26 to 30	51	35	68.6

PERIOD OF INCUBATION OF THE VIRUS IN THE LEAFHOPPER

The term "incubation period" as used in this paper refers to the period from the time the leafhopper acquires the virus to the time that it is able to inject enough virus into a plant to produce infection. To determine the length of this period and to gain information regarding the rate of increase in the ability of leafhoppers to cause infection after the acquisition of the virus, an experiment involving a rather extensive series of leafhopper transfers was planned. Young adult leafhoppers, which had been starved 14 to 16 hours, were caged singly and placed on curly top beets at a temperature of approximately 100° F. After 1 hour they were removed and immediately placed on healthy seedling beets. Once each hour for the next 11 hours they were transferred to fresh beets. After the twelfth hour they were transferred to fresh beets at 12-hour intervals for the next 6½ days. Controls were left on diseased beets for 6 days and transferred to healthy plants at 12-hour intervals beginning the seventh day. The last two transfers of the first lot of leafhoppers coincided with the two transfers of the control leafhoppers. Therefore, as a preparation for infecting on the seventh day, the first lot had 1 hour on a diseased plant and eleven 1-hour periods and eleven 12-hour periods on healthy plants; whereas the controls had 6 days on a curly top plant.

Table 4 shows the individual performance of each of 20 leafhoppers on each of the 24 healthy plants exposed to feeding during the 7-day period. The results of nine such experiments are summarized in table 5. Under the conditions of these tests, the minimum incubation period of the virus in the leafhopper was 4 hours. The ability of the leafhoppers to transmit virus increased appreciably during the first 12 hours, but there is no evidence that there was any further increase in infective ability during the following 6½ days.

TABLE 4.—*Infection produced by leafhoppers in successive transfers on seedling beets over a period of 7 days following a 1-hour feeding period on a curly top beet*

Duration and serial No. of feeding period	Plants infected [1] by leafhopper No.—																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1-hour periods:																				
2 [2]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
6	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
8	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
10	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
12-hour periods:																				
13	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+
14	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
15	+	−	−	+	+	−	−	+	−	−	−	+	−	−	+	+	−	−	+	+
16	+	−	−	+	−	−	−	−	−	−	−	+	−	+	+	−	−	+	+	+
17	−	−	−	+	−	−	−	−	−	−	−	+	+	−	+	−	−	−	+	+
18	+	−	+	+	+	−	+	−	−	−	−	+	−	+	+	+	−	−	+	+
19	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	+	+	+
20	+	−	+	−	−	+	−	−	−	−	+	+	−	−	+	+	−	+	+	+
21	−	−	−	+	−	−	−	−	−	+	+	+	−	+	+	−	+	−	−	+
22	−	−	−	+	−	−	−	+	−	+	+	+	+	+	−	−	+	+	+	+
23	−	−	−	+	−	+	+	−	−	−	+	+	+	−	−	−	−	−	−	+
24	+	−	−	+	+	−	−	−	−	−	+	+	+	−	+	+	−	−	+	+
25	−	−	−	+	−	−	−	−	−	+	+	+	−	+	−	−	−	+	+	+

[1] Plus (+) and minus (−) signs indicate diseased and healthy plants respectively.
[2] Feeding period No. 1 was on a curly top beet.

TABLE 5.—*Infection produced by leafhoppers in successive transfers on seedling beets over a period of 7 days following a 1-hour feeding period on a curly top beet* [1]

Duration and serial No. of feeding period	Plants infected									
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6	Experiment 7	Experiment 8	Experiment 9	Total
1-hour periods:	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	1	0	0	1	0	1	0	0	5	2.8
5	1	0	1	1	0	0	2	1	2	8	4.4
6	0	1	1	0	0	0	0	2	1	5	2.8
7	3	0	2	1	0	0	0	1	3	10	5.6
8	3	1	1	4	2	1	1	1	5	19	10.6
9	1	1	2	2	3	0	0	3	0	12	6.7
10	2	3	1	2	1	2	1	2	3	17	9.4
11	1	5	0	4	5	1	1	1	1	19	10.6
12	4	8	1	5	1	1	0	1	2	23	12.8
12-hour periods:										
13	5	5	8	6	10	4	3	8	4	53	29.4
14	6	4	8	6	7	8	3	9	4	55	30.6
15	2	4	5	4	3	4	3	8	10	43	23.9
16	7	5	8	6	7	10	3	9	9	64	35.6
17	4	4	5	4	8	6	4	7	7	49	27.2
18	6	6	8	6	5	11	1	8	13	64	35.6
19	9	3	9	3	8	8	2	9	7	58	32.2
20	------	------	7	5	7	7	1	8	11	46	32.9
21	------	------	6	3	6	8	1	6	9	39	27.9
22	------	------	7	4	3	7	2	10	13	46	32.9
23	------	------	4	4	6	3	2	6	7	32	22.9
24	------	------	7	5	7	4	4	6	10	43	30.7
25	------	------	7	7	11	3	2	3	7	40	28.6
Checks: [2]										
24	------	------	7	14	14	5	8	7	11	66	47.1
25	------	------	6	16	13	0	8	5	5	53	37.9

[1] 20 leafhoppers were used in each experiment.
[2] These leafhoppers fed on curly top beets over a period of 6 days and were then transferred to seedling beets at 12-hour intervals.

In each of the nine experiments there was a striking variation in the amount of infection produced by the different individuals of the group. This is illustrated in table 4. Leafhopper No. 4 produced infection in the fifth hour, failed to produce infection in only two of the remaining 1-hour feeding periods, and failed to produce infection in only three of the 12-hour feeding periods; whereas leafhopper No. 6 infected only two plants in 7 days and these were in the 12-hour feeding periods. This type of performance is apparently the result chiefly of characteristics of individual leafhoppers and is not due necessarily to a variation in the amount of virus originally picked up by the leafhoppers, since similar variations are obtained when leafhoppers reared on curly top plants are used in successive short-interval transfers.

Whether the slightly greater amount of infection by the check group of leafhoppers on the seventh day is of significance is difficult to determine. A factor which cannot be evaluated and which may have influenced results is the relative number of leafhoppers in the two groups that actually carried virus. Of the 140 leafhoppers used in the frequent transfers, 32 produced no infection. If it were assumed that these 32 obtained no virus in the 1-hour period of feeding on diseased beets and that all the leafhoppers allowed to feed on diseased beets for 6 days obtained virus, part of the difference in infection might be accounted for. Even without any correction for this factor, it is evident from the totals shown in table 5 that the check leafhoppers were superior only to a small degree to those of the frequently transferred lot in ability to infect on the seventh day.

Evidence as to the relative amounts of virus carried by the two lots of leafhoppers at the end of 7 days was obtained in four of the nine tests by removing each lot of 20 leafhoppers and testing for virus content by the alcoholic-precipitation method already described. Five of the eighty plants inoculated from the material derived from the frequently transferred leafhoppers were infected; whereas 24 of the 80 plants inoculated from material derived from the control leafhoppers were infected. This is strong evidence of a greater virus content in the control group, and it shows that ability to infect is not always proportional to the amount of virus carried in the leafhoppers.

EFFECT OF LENGTH OF FASTING PERIOD ON INFECTION IN SHORT-TIME FEEDINGS

The effect of a period of fasting on ability of leafhoppers to produce infection was noted in a series of tests made in connection with the study of variation in infective ability discussed in a later section. The viruliferous leafhoppers to be tested were starved overnight. Each leafhopper was then allowed to feed 6 minutes on each of 20 seedling beet plants as rapidly as it would complete its feedings. The beginning and ending of each feeding period were recorded, thus making available a complete record of the feeding time and the fasting interval for each of the 20 feedings of each leafhopper. The records of 209 leafhoppers are available from these experiments.

When all the feeding tests were completed, the data were assembled on the basis of the percentage of infection produced in each of the 20 successive feedings. As shown in figure 1, where these data are plotted, infection was relatively low in the first feeding period, some-

what greater in the second, and rose in the third to a level that was maintained rather consistently for the succeeding 17 periods.

The percentage of infection was calculated also on the basis of the length of the period of fasting prior to feeding, in an effort to determine the effect of fasting periods of different duration on infection in the subsequent 6-minute feeding. The fasting periods varied from less than 1 minute to more than 18 hours. The data for a total of 4,045 fasting periods were arranged in 21 groups, as follows: 10 groups with fasting periods increasing by 1-minute intervals, respectively, from 1 to 10 minutes; 4 groups with fasting periods increasing by 5-minute intervals, respectively, from 15 to 30 minutes; 6 groups with fasting periods increasing by 1-hour intervals, respectively, from 1 to 6 hours; 1 group with a fasting period of 18 hours.

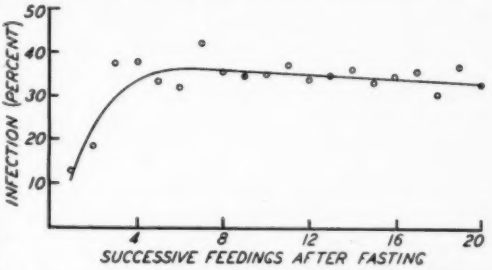

FIGURE 1.—Infection of seedling beets caused by viruliferous leafhoppers in 20 successive 6-minute feedings following an initial 16- to 20-hour fast period.

The results are plotted in figure 2, the percentages of infection being shown on the ordinate axis. These points represent averages of infections based on from 4 to a maximum of 475 cases. Fasting period is plotted on the abscissa axis as the logarithm of the time in minutes. There is some indication from the data that when the leafhoppers fast only 1 to 3 minutes before feeding they are less effective vectors than when they fast 15 minutes to 3 hours before feeding. Infections secured following an 18-hour fasting period were extremely low.

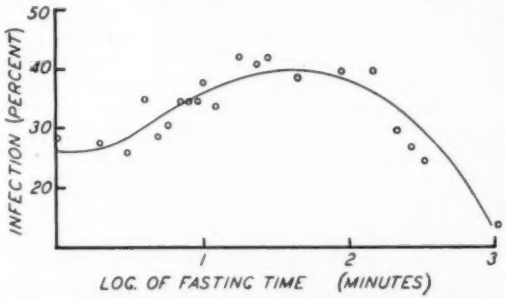

FIGURE 2.—Effect of length of the fasting period immediately before inoculations were made with viruliferous beet leafhoppers on the infections secured with seedling sugar beets in a subsequent 6-minute feeding period.

Little is known regarding the factors that produce these results. The failure of leafhoppers to produce the normal amount of infection in the first and second 6-minute feedings after a long fasting period can hardly have been due to a weakened condition of the leafhoppers, for they were quite active and apparently in good physical condition. The manner in which they fed varied in no way from that in later feedings, so far as known. It is suggested, however, that starvation,

combined with a certain amount of desiccation, may have brought about certain changes that tended to inactivate any virus that might have been held in the salivary glands; hence the usual amount of infection could have been produced only after these conditions were corrected.

The greater amount of infection produced in the feedings that followed fasting periods of 15 minutes to 3 hours may have resulted from the injection of greater amounts of saliva into the plant in feedings following the fast, or it may have come from an increase in virus content of the salivary glands by accretion from the blood during the fast, or it may have been due to other factors. However, an increase in virus content of the salivary glands by virus multiplication during the fast would seem to be excluded in view of evidence presented later in this paper which indicates that no multiplication of virus occurs in the leafhopper.

DISTRIBUTION OF VIRUS IN DIFFERENT PARTS OF THE LEAFHOPPER

The determination of the distribution and location of the virus in the beet leafhopper was only partially accomplished, because of the difficulty of avoiding contamination from parts other than those selected for test. Tests to gain some concept of the virus content of the blood, salivary glands, alimentary tract, and feces were made as follows:

Blood.—Leafhoppers were etherized and fastened to a piece of cardboard by means of a thread drawn tight enough to place a slight pressure on the thorax. Under a dissecting microscope, a small opening was made in the abdominal wall, through which a small drop of liquid escaped. This coagulated in a short time and was removed on the point of a needle to a 5-percent sugar solution. Drops of blood from 20 leafhoppers were mixed with 1 cc of sugar solution, and 20 nonviruliferous leafhoppers were allowed to feed on the mixture. The leafhoppers were then caged singly on seedling beets to determine whether they had picked up virus. A wash of alcoholic precipitate of the macerated remains of the leafhoppers from which the drops of blood were taken was tested for virus and a further test was made of the virus content of 20 viruliferous leafhoppers from which no blood was removed.

Salivary glands.—The heads with part of the thorax were removed from 20 viruliferous leafhoppers. The salivary glands were dissected out in drops of water, passed through distilled water, and macerated in 1 cc of sugar solution, and 20 nonviruliferous leafhoppers were allowed to feed on the mixture. As a check, the remains of the dissected leafhoppers and also 20 nondissected leafhoppers were tested by the alcoholic-precipitation method.

Feces.—Large numbers (200 to 300) of viruliferous leafhoppers were placed on curly top diseased leaves in large Erlenmeyer flasks and allowed to feed at a temperature of 85° to 110° F. for 3 to 4 hours. Air was circulated to keep the flasks dry. The feces deposited on the sides of the flasks were removed by washing with distilled water. Alcohol was added and a wash of the precipitate was prepared and tested for virus. An extract from 20 viruliferous leafhoppers was tested as a check.

Alimentary canal.—Viruliferous leafhoppers were caged on petioles of healthy beets for 24 hours in order to allow for partial or complete removal of materials previously taken up from diseased plants. After this period the alimentary tracts from 20 leafhoppers were dissected out in drops of distilled water. The alimentary tracts were then passed through distilled water and macerated in sugar solution. The Malpighian tubules were included with the tested parts, and undoubtedly some blood was carried over. The remains of the dissected leafhoppers and also 20 nondissected leafhoppers were tested as checks.

The results of these tests (table 6) show that virus was obtained from all of the parts tested. Probably the results from blood and feces may be considered the most reliable, since the blood and feces had least chance of being contaminated with virus from other parts. These results indicate that a small amount of virus passed through the leafhopper and was present in active condition in the feces. There is apparently much less virus in the salivary glands than in the remainder of the leafhopper. This seems to show that the salivary glands are not the virus reservoirs of the insect.

TABLE 6.—*Virus content of different parts of the beet leafhopper*

Experiment No.	Source of inoculum	Plants inoculated	Plants infected	
		Number	*Number*	*Percent*
1	Blood	60	14	23.3
	Leafhoppers (remains)	60	21	35.0
	Leafhoppers	60	23	38.3
2	Salivary glands	40	10	25.0
	Leafhoppers (remains)	40	28	70.0
	Leafhoppers	40	26	65.0
3	Feces	148	18	12.2
	Leafhoppers	140	87	62.1
4	Alimentary canal	40	18	45.0
	Leafhoppers (remains)	40	22	55.0
	Leafhoppers	40	17	42.5

DOES THE VIRUS MULTIPLY IN THE INSECT VECTOR?[3]

The manner of transmission of plant viruses by insects undoubtedly varies, depending on the species of insect involved and perhaps on the virus also. Certain insects transmit apparently by simple transfer of the virus on the mouth parts. In such cases it is probably unnecessary for the virus to pass through the body of the insect. Vectors of this type soon lose their power to cause infection when deprived of new sources of virus.

Other insects ingest virus and retain it in their bodies over more or less extended periods, as is shown by their ability to transmit disease after being confined to immune plants. Retention of virus in this way has led to the suggestion of a biological relation between virus and insect in certain instances. That this relationship involves a stage of development of the virus in the insect has not been shown, but it has been rather generally accepted as evidence that certain viruses multiply in their insect vectors.

Kunkel (*9*) suggested that the long incubation period of the virus of aster yellows in the vector *Cicadula sexnotata* Fall and the length

[3] After this paper was prepared for publication, Freitag (*6*) published an article in which extensive evidence is presented indicating that the curly top virus does not multiply in the insect vector.

of time it retains the virus when confined to immune plants indicate multiplication of the virus in some tissue of the insect.

In studies on *Cicadulina mbila* Naude, vector of the virus of streak disease of maize, Storey (*19*) developed "active" races of the insect, capable of causing infection after feeding on diseased plants, and "inactive" races, incapable of causing infection after feeding on diseased plants. When transferred to virus-free plants at frequent intervals, active individuals usually retained the virus during life but certain individuals lost it. Leafhoppers that had lost the virus regained their ability to produce infection after feeding on diseased plants. Inactive individuals were able to act as vectors when virus was introduced into their body cavities by artificial means. However, these leafhoppers retained their ability to transmit for only a relatively short period. Apparently the size of the initial charge of virus greatly influenced the length of time in which the leafhopper could produce infection. Therefore, it seems probable in this case that there was no multiplication of virus in the insect.

Fukushi (*8*) found that the virus of dwarf disease of rice is carried through the egg stage to the third generation in the leafhopper *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl. This probably is the strongest evidence available that a plant virus multiplies in its insect vector, for in the absence of multiplication infection from enormous dilutions would be required.

Severin (*11*) found that male beet leafhoppers when transferred daily to healthy sugar beets retained their ability to produce infection throughout their adult life. Other evidence supports the view that after leafhoppers acquire the virus of curly top they retain their ability to produce infection for long periods under a wide range of conditions.

A number of experiments were performed to determine whether this ability is due to multiplication of the virus in the insect or to a long period of retention of the virus acquired by the insect through feeding.

In one series of tests attempts were made to introduce the virus into the blood of the leafhopper in a manner similar to that employed by Storey (*20*) in introducing the virus of maize streak into *Cicadulina mbila*. In these tests nonviruliferous leafhoppers were etherized and bound to a piece of cardboard under a binocular microscope. With the aid of a micromanipulator, the tip of a capillary tube filled with a medium containing the curly top virus was inserted into the abdominal cavity and a portion of the liquid injected into the leafhopper. Fifty leafhoppers were treated in this manner and caged on seedling beets. Mortality among the leafhoppers was high and after 7 days only seven were alive. No infection was obtained with the seedling beets.

In other tests, punctures through drops of media containing the virus were made in the abdominal and thoracic cavities of nonviruliferous leafhoppers by means of small needles. Phloem exudate from diseased beets and drops of blood from viruliferous leafhoppers were used as sources of virus. One hundred leafhoppers were treated and caged singly on seedling beets. Mortality among these leafhoppers was much lower than among those injected by means of capillary tubes. After 7 days, 51 leafhoppers were alive. No infection was obtained on the test beets.

To determine whether the virus content of the leafhoppers decreased with feeding on plants from which they could obtain no additional

virus, viruliferous leafhoppers were confined to plants very resistant or immune to curly top and tested at intervals for ability to infect seedling beets and for virus content.

In the earlier tests, Australian saltbush (*Atriplex semibaccata* R. Br.) was selected as the resistant plant on which to confine the leafhoppers. Although extremely resistant, this plant is probably not immune to curly top under all conditions, for Severin and Henderson (*15*) succeeded in obtaining a small amount of virus from 3 of 30 plants previously inoculated repeatedly by means of large numbers of viruliferous leafhoppers. In all tests reported here, large numbers of the nymphal progeny of the colonies on Australian saltbush were caged on seedling beets at intervals during the course of the test to determine whether they had acquired virus. None produced infection, and it was accordingly assumed that the adult leafhoppers of the colonies had not acquired virus from the plant. After Severin and Freitag (*14*) showed that sweet corn is a favorable food plant for the leafhopper and also immune to curly top, all experiments were repeated with the Golden Bantam variety of sweet corn used in place of Australian saltbush. No significant difference was obtained with leafhoppers confined on the two species, and it seems probable that the results obtained with Australian saltbush are as reliable as those obtained with corn.

Three tests were made. In the first test, colonies of viruliferous leafhoppers reared to the adult stage on curly top beets were divided into two lots. One lot remained on curly top beets and the other lot was transferred to Australian saltbush or sweet corn. Tests of the ability to produce infection were made at weekly intervals by caging, singly, 20 leafhoppers on seedling beets for 24 hours. After 24 hours, the 20 leafhoppers of each lot were macerated and tested for virus content by the alcoholic-precipitation method already described. The results are summarized in experiment 1 of table 7.

In the second test, colonies of approximately 1,000 nonviruliferous leafhoppers just emerged into the adult stage were placed on curly top plants for a period of 2 hours at a temperature of about 110° F. This short feeding period was used in order to provide a smaller charge of virus than that held by leafhoppers reared on diseased plants. At the expiration of the 2-hour feeding period each colony was divided into two lots. One lot was placed on Australian saltbush or sweet corn and the other lot remained on curly top beets. At intervals, 20 leafhoppers were taken from each lot and caged singly on seedling beets for 24 hours. The leafhoppers were then macerated and each lot tested for virus content as in the first experiment. The results are summarized in experiment 2 of table 7.

In the third test nonviruliferous leafhoppers were allowed a feeding period of 6 hours on a water wash of an alcoholic precipitate from macerated viruliferous leafhoppers. Previous tests had shown that leafhoppers acquire very low charges of virus through feeding on such suspensions. After feeding on the liquid the leafhoppers were caged on sweet corn. At intervals, 20 leafhoppers were removed and caged 7 days on seedling beets to determine their ability to infect. At the same time 20 other leafhoppers were removed from the cage and tested for virus content by the alcoholic-precipitation method already described. Checks were run with leafhoppers from curly top beets as in other experiments. The results of several repetitions of this experiment are shown in experiment 3 of table 7.

TABLE 7.—*Influence of size of initial virus charge on the decrease in infective ability and virus content of leafhoppers deprived of new sources of virus by being confined to resistant or immune plants*

Experiment No.	Way in which leafhoppers acquired virus	Inoculations[1] on seedling beets by means of—	Plants on which leafhoppers were kept until tested on seedling beets	Infection[2] on seedling beets after—										
				0 week[3]	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks
				Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	4 weeks feeding on curly top beets.	Direct feeding, 24 hours	Saltbush or corn	62	67	52	63	62	55	51	52	72	------	------
			Curly top beet	76	63	73	71	73	76	83	50	80	------	------
		Extracts from leafhoppers used in test.	Saltbush or corn	45	38	13	3	5	12	11	2	5	------	------
			Curly top beet	43	46	68	68	66	67	66	60	60	------	------
2	2 hours feeding on curly top beets.	Direct feeding 24 hours	Saltbush or corn	32	67	76	46	45	38	28	32	21	25	------
			Curly top beet	36	53	61	68	71	51	50	52	48	70	------
		Extracts from leafhoppers used in test.	Saltbush or corn	21	26	3	5	3	6	0	7	2	0	------
			Curly top beet	21	45	28	42	68	45	30	42	47	55	------
3	6 hours feeding on extracts from viruliferous leafhoppers.	Direct feeding, 7 days	Saltbush or corn	43	------	29	------	14	------	7	------	2	------	0
			Curly top beet	38	------	91	------	96	------	89	------	93	------	86
		Extracts from leafhoppers from the same sources as those used in test.	Saltbush or corn	1	------	0	------	0	------	0	------	0	------	0
			Curly top beet	0	------	42	------	36	------	31	------	47	------	32

[1] Leafhoppers were caged singly on seedling beets 24 hours in experiments 1 and 2. The resulting infection is considered a relative measure of their ability to infect. An extract from these leafhoppers was then prepared and fed to nonviruliferous leafhoppers. These were caged singly on seedling beets 7 days. The resulting infection is considered a relative measure of the virus content of the leafhoppers from which the extract was derived.

[2] In experiments 1 and 2, 160 plants were inoculated in each test at each time interval. In experiment 3 the number of inoculated plants per test ranged from 40 to 180.

[3] These tests were made immediately following the feeding period in which the test leafhoppers acquired their charge of virus.

An additional test was made in which leafhoppers were transferred at daily intervals to healthy beets. In this test 28 half-grown nonviruliferous nymphs were allowed to feed on a virus suspension, and 6 hours later they were caged singly on healthy beets having four to six leaves. The leafhoppers were transferred daily to fresh beets over a period of 90 days or until they died. Infection decreased with time and no infection was produced by any of these leafhoppers after 54 days. The record of five leafhoppers for 90 days is shown in table 8. On the ninety-first day these leafhoppers were allowed to feed on a curly top plant and were then transferred daily to healthy beets over a period of 9 days. Three of these leafhoppers acquired virus and infected a large percentage of the plants on which they fed. This proves conclusively that these leafhoppers had not lost their ability to act as vectors and that their previous inability to infect was due to depletion of their virus content.

TABLE 8.—*Plants infected*[1] *in 90 successive days by five leafhoppers that had previously acquired a low charge of virus by feeding 6 hours on extracts from viruliferous leafhoppers; also plants infected by the same leafhoppers after feeding 1 day on a curly top plant*

Day No.	Infection by leafhopper No.—					Day No.	Infection by leafhopper No. —				
	1	2	3	4	5		1	2	3	4	5
1	+	−	+	−	+	30	−	−	−	−	+
2	−	−	+	−	+	31-33	(2)	(2)	(2)	(2)	(2)
3	−	−	+	−	+	34	+	+	+	−	+
4	−	−	−	−	+	35	+	−	−	−	−
5	−	−	−	−	−	36	+	−	−	−	−
6	−	−	−	−	+	37	−	−	−	−	−
7	+	−	−	−	+	38	−	−	−	−	+
8	+	−	−	−	+	39	−	−	+	−	−
9	−	−	−	−	+	40	−	−	−	−	+
10	−	−	+	−	+	41	−	−	−	−	+
11	−	−	+	−	+	42	−	−	−	−	−
12	−	−	−	−	+	43	−	−	−	−	+
13	−	−	+	−	+	44	−	−	−	−	−
14	−	−	+	−	+	45	−	−	+	−	+
15	−	−	−	−	−	46	−	−	−	−	+
16	−	−	−	−	+	47-52	(2)	(2)	(2)	(2)	(2)
17	−	−	−	−	−	53	−	+	−	−	−
18	−	−	+	−	+	54	−	−	−	−	+
19	−	+	−	−	+	55-90	(2)	(2)	(2)	(2)	(2)
20	−	−	−	−	−	91	(3)	(3)	(3)	(3)	(3)
21	−	−	−	−	+	92	+	−	−	−	+
22	−	−	−	−	+	93	+	−	+	−	+
23	−	−	−	−	+	94	+	−	+	−	+
24	−	−	−	−	+	95	+	−	+	−	+
25	−	−	−	−	−	96	+	−	+	−	+
26	−	−	−	−	−	97	+	−	−	−	−
27	−	−	−	−	+	98	−	−	+	−	+
28	−	−	−	−	+	99	+	−	−	−	+
29	−	−	+	−	+	100	+	−	+	−	+

[1] Plus (+) and minus (−) indicate diseased and healthy plants, respectively.
[2] No plants were infected on these days.
[3] The leafhoppers fed on a curly top plant during this day.

The results of these experiments showed that when leafhoppers were transferred at frequent intervals to beets or were confined to plants extremely resistant or immune to curly top their virus content decreased with time. The decrease in virus content was more rapid than the decrease in ability to produce infection by direct feeding, which is further evidence that the amount of virus required by the leafhopper for maximum ability to infect is appreciably less than the maximum charge of virus that it is capable of carrying. The level

to which the virus content and ability to infect dropped in 8 to 10 weeks was influenced by the size of the charge of virus carried by the leafhoppers at the time they were placed on immune plants.

In view of the foregoing evidence and the failure to impart ability to infect by injecting virus suspensions into the leafhoppers, it seems probable that there is no multiplication of the curly top virus in the beet leafhopper. The prolonged periods over which the leafhopper is able to produce infection when deprived of plant sources of virus may be explained by the ability of the virus to retain its activity in the body of the leafhopper for long periods and to a slow rate of loss of virus by the leafhopper. The period of virus retention is surprisingly long.

INDIVIDUAL VARIATION IN ABILITY TO TRANSMIT THE VIRUS

From results already presented it is evident that individual beet leafhoppers vary greatly in their ability to transmit the curly top virus. Similar individual variations in infective ability have been found in at least two other vectors of plant viruses.

Storey (*19*) found individuals of *Cicadulina mbila* incapable of transmitting the virus of streak disease of maize under the conditions of his experiments. Selection and mating in successive generations produced races in which none of the individuals were capable of transmitting virus. Selection and mating also gave races in which all the individuals were able to transmit virus. Tests of progeny of crosses between the two races indicated that ability to transmit is a sex-linked dominant character. Fukushi (*7*) observed a wide variation in the ability of *Nephotettix apicalis* var. *cincticeps* to transmit the virus of stunt disease of rice and found numerous individuals apparently unable to transmit it even after they had fed throughout their nymphal stages on infected plants.

By testing large numbers of beet leafhoppers from stock colonies and field collections individuals were found that failed to transmit the curly top virus to small beets in 7 to 14 days in sufficient quantities to cause infection. However, when successive selections and matings were made from these leafhoppers their F_3, F_5, and F_7 progeny retained the ability to transmit virus when caged 14 days on seedling beets. These tests were extensive enough to indicate strongly that, in the colonies used, there were no individuals inherently lacking in ability to transmit the virus. It was not determined whether these selections were less effective than the original stock in transmitting virus in short-interval transfers.

An attempt was made by successive selection and mating to develop strains of leafhoppers that would differ in ability to transmit the virus. In these tests individuals were allowed to feed 6 minutes successively on each of 20 seedling beets. The percentage of infection resulting was considered a measure of the transmissive ability of the leafhopper. Individuals that produced little or no infection in these tests were mated, and selections were made from the progeny in each successive generation through the F_7 generation. A similar process of breeding and selection was carried through the F_6 generation for leafhoppers high in ability to transmit. At first selections were made of the most promising individuals regardless of source, but later close inbreeding in each line was practiced and only brother and sister matings were

made. Tests of the progeny of each generation were made as described, except in the F_5, F_6, and F_7 generations of the low strain. Here, in order to give the leafhoppers a greater chance to produce infection, they were left on the seedling beets 12 to 24 hours.

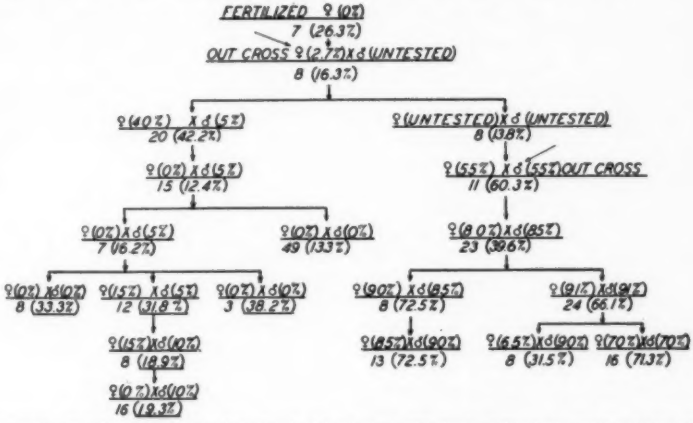

FIGURE 3.—Results of selecting and mating beet leafhoppers differing in ability to transmit curly top virus. In the diagram the percentage shown above the line following the symbol for sex shows the infective capacity found. The first number below the line indicates the number of leafhoppers in the progeny of this mating that were tested. The second number (in parentheses) indicates the average percentage of infection produced. Percentages of infection were determined by the number of plants infected when 20 plants were inoculated. Six-minute feedings were used except in the tests of progenies of the last three generations of the low strain (numbers underlined), in which the feeding periods were longer (12 to 24 hours).

These tests were not carried as far as was desirable and the original selections were not as rigid as they should have been, especially in the strain selected for high ability to transmit. The two lines of descent, including the individuals that were mated and the results of the tests of the progeny of each mating, are shown in figure 3.

Figure 4 shows a graphic analysis of the entire tested populations of each strain from the third generation to the end of the selections, based on the number of plants each leafhopper infected out of the 20 plants on which it fed. Figure 4 includes all the leafhoppers from figure 3 and several more from each strain which for various reasons were not included in figure 3. The data in figure 4 show that the leafhoppers of the low strain were predominantly low in ability to transmit and that the leafhoppers of the high strain were predominantly high in ability to transmit.

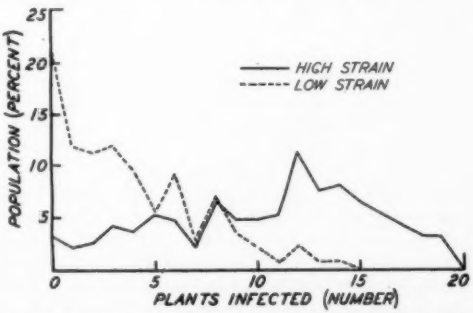

FIGURE 4.—Distribution of the total tested populations of the high and low strains (beginning with the third generation) on the basis of individual ability of leafhoppers to transmit curly top virus as indicated by the number of plants infected by each individual in the tests in which each leafhopper fed successively on each of 20 seedling beets.

The individuals of two populations shown in figure 4 were divided into two groups. Those individuals that infected seven plants or less were considered low in ability to transmit and those that infected eight plants or more were considered high in ability to transmit. On this basis, 119 of the 143 tested individuals of the low strain were low in ability to transmit and 24 individuals were high. In the high strain, 53 of the 186 individuals were low in ability to transmit and 133 were high.

Using these data to determine χ^2 according to Fisher's (*5*) method for testing for independence in a 2×2 classification, a value of 97.038 was obtained. Since a value of 6.635 gives odds of 100 to 1 favoring independence, a χ^2 value of 97.038 would give very high odds in favor of the concept that the two strains of leafhoppers used in these experiments were genetically different.

The foregoing results seem to indicate clearly that degree of effectiveness in transmitting curly top virus is heritable and that strains of leafhoppers below the average and strains above the average in transmissive ability can be developed. As yet, however, there is no evidence that a strain inherently lacking in ability to transmit the curly top virus can be developed.

RELATION OF CURLY TOP VIRUS TO CERTAIN NONVECTOR INSECTS

The only vector of the curly top virus in North America is the leafhopper *Eutettix tenellus*. None of the other species of insects that feed on sugar beets or other plants susceptible to curly top have been found capable of transmitting the virus, even when used in very large numbers. Several of the species of insects that feed on beets seek out the phloem and must of necessity pick up virus in the course of their feeding since the virus content of the phloem is high (*2*). The relations of of the virus to these insects and the factors preventing their acting as vectors hold considerable interest for those dealing with virus diseases.

ACQUISITION OF VIRUS

A number of the common species of insects that feed on sugar beets were tested for virus content after being allowed to feed on curly top beets. These species [4] include the aphids *Myzus persicae* (Sulz.), *Aphis rumicis* L., and *Pemphigus betae* Doane; the leafhoppers *Aceratagallia californica* (Baker), *Phlepsius strobi* (Fitch), and *Empoasca solana* (De Long); the thrips *Hercothrips femoralis* (Reuter); and a red spider, *Tetranychus* sp. Freehand and prepared sections of beet leaves have demonstrated that *Aphis persicae* and *Aceratagallia californica* are phloem feeders. Probably *Aphis rumicis*, *Pemphigus betae*, and *Phlepsius strobi* also feed on the phloem. The remaining three species probably obtain all or most of their food from the parenchyma.

The insects were caged on diseased beets 2 days to 2 weeks, the time varying with the species of insect involved. At the expiration of the feeding period, a 30-mg sample of insects was taken. An extract was prepared from this in the manner already described, and

[4] The writers are indebted to P. W. Oman for identification of *Empoasca solana* and *Aceratagallia californica;* to J. C. Crawford and Stanley F. Bailey for identification of *Hercothrips femoralis;* to H. E. Ewing for identification of *Tetranychus* sp.; and to P. W. Mason for identification of *Myzus persicae.*

fed to nonviruliferous beet leafhoppers. After a feeding period of 4 to 6 hours on the prepared extract, the leafhoppers were caged singly on seedling beets for 7 days. The number of plants infected was taken as a measure of the virus content of the insects from which the extracts were derived. The virus content of the leaves of the diseased beets on which the insects had fed was tested in the usual way by the alcoholic-precipitation method, 1 cc of juice being used in each test.

All the species of insects used in these tests picked up virus from diseased beets, and some of them acquired it in relatively large quantities. One might judge that the virus per unit weight is more concentrated in some of these insects than in the beet juice. It is worthy of note that those species that presumably fed only on the parenchyma acquired virus, although apparently the quantity was much smaller than that obtained by insects that fed on the phloem. No method has been devised for determining the original tissue source of the virus picked up by insects that restrict their feeding to parenchyma. While it may have been picked up directly from the parenchyma cells, it seems more probable that it was derived from phloem exudate that had escaped into the intercellular spaces of the parenchymatous tissues. The results of these tests are shown in table 9.

TABLE 9.—*Acquisition of virus by different species of insects*

Insect tested	Source of virus and results					
	Extracts from macerated insects			Extracts from juice of plants on which insects fed		
	Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Aceratagallia californica	140	62	44.3	100	42	42.0
Phlepsius strobi	20	5	25.0			
Myzus persicae	100	61	61.0	20	15	75.0
Aphis rumicis	100	23	23.0	100	31	31.0
Pemphigus betae	60	9	15.0	20	12	60.0
Empoasca solana	180	37	20.5	180	69	38.3
Hercothrips femoralis	220	22	10.0	160	70	43.8
Tetranychus sp. (from beet)	60	17	28.3	60	28	46.7
Tetranychus sp. (from oxalis)	80	11	13.8	80	0	.0

LONGEVITY OF VIRUS IN NONVECTOR SPECIES

With the exception of the aphid *Pemphigus betae*, the species of insects mentioned above were used in tests to determine the time the virus remained active in the insects after they were removed from diseased plants. Large numbers of individuals of each species were caged on curly top beets for 2 days to 2 weeks, after which they were transferred to healthy beet plants. At intervals, 30-mg quantities were removed and tested for virus content. The two species of aphids multiplied so rapidly that it was difficult to keep the individuals that had fed on diseased plants separate from their progeny. After a few preliminary trials, attempts to effect this separation were abandoned and tests were made on 30-mg samples from each colony without regard to age of insects used. With all other species, only the individuals that fed on diseased plants entered into the tests for the presence of virus.

Myzus persicae retained virus 14 days and *Aphis rumicis* retained it 7 days. It is probable that virus could have been recovered from these two species after longer periods on healthy plants if tests had been restricted to the insects that fed on diseased plants. *Aceratagallia californica* retained virus 21 days, though there was a progressive drop in virus content from the time the leafhoppers were removed from diseased plants. Those species that fed only on parenchyma lost the virus in a relatively short time. The results of these tests are shown in table 10.

TABLE 10.—*Retention of virus by different species of insects*

Insect tested	Plants inoculated	Plants infected from extracts of insects after the insects had been on healthy beet plants for—					
		0 days [1]	1 day	3 days	7 days	14 days	21 days
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Aceratagallia californica	80	35	21	18	9	2	1
Phlepsius strobi	20	8	0	0	0	----	----
Myzus persicae	40	24	22	20	9	2	0
Aphis rumicis	100	25	22	16	11	0	----
Empoasca solana	80	22	10	0	0	----	----
Hercothrips femoralis	20	1	0	0	0	----	----
Tetranychus sp	60	5	2	1	0	----	----

[1] Tests made immediately after removal of the insects from curly top plants.

DISCUSSION

The curly top virus extracted from the plant by the beet leafhopper in its feeding is probably derived chiefly from the phloem. As the leafhopper feeds, the virus evidently passes through the mouth parts into the alimentary canal along with food materials. Little is known regarding the effect of the contents of the alimentary canal on the virus. The experiments reported here show that the virus passed through the insect and was recovered in the feces. The concentration of virus in the feces could not be determined, but there is reason to suspect that it was far below that of the food material that the leafhopper ingested. Experiments by Severin (*12*) indicate that under certain conditions no virus is recoverable from the feces. It seems probable, therefore, that there may be considerable inactivation of virus in the alimentary tract. Studies with dyes indicate that at high temperatures ingested materials pass through the leafhoppers in 30 minutes to 2 hours. Therefore, if there is inactivation of virus in the alimentary tract, the inactivating substances must be quite effective and capable of acting in a relatively short time.

The virus passes from the food material taken in by the leafhopper, through the walls of the alimentary canal, into the blood, where, according to present indications, it reaches a maximum concentration after the leafhopper has fed on diseased plants for 7 days or more.

The blood would appear to be a very favorable medium for preserving the virus. In reaction it is slightly alkaline (*2*), in this respect being somewhat similar to phloem content, which is considered the most favorable plant medium for the virus. The high colloidal content of the blood may function in greatly reducing loss by diffusion.

Since the curly top virus is able to retain its activity for 4 weeks or more in certain extracts from plant juice and for 10 months (*2*) or

more in dried phloem content, it seems probable that it would retain its activity for relatively long periods in a medium as favorable as the blood is presumed to be. Once the virus enters the blood, loss would be restricted to that resulting from inactivation and from diffusion into the salivary glands and possibly back into the alimentary tract. Assuming no multiplication of virus in the insect, the time that a leafhopper would remain viruliferous when deprived of new sources of virus would depend, therefore, on the original amount of virus carried, on rate of inactivation, and on rate of loss by diffusion into the salivary glands and into the alimentary tract. If the initial virus charge was high and losses were small, the leafhopper would retain its ability to transmit virus over a relatively long period.

Of the factors influencing retention of virus by the leafhopper, only size of initial charge can be governed experimentally. Decreasing the size of the initial virus charge definitely shortened the time in which the leafhoppers were able to produce a maximum amount of infection. This, together with the evidence that the virus content decreased with time regardless of the size of the initial charge, indicates lack of reproduction of the virus in the leafhopper. If the same condition holds generally among insect vectors that retain viruses for long periods, much of the evidence now accepted as supporting the idea that certain plant viruses multiply in their insect vectors must be reevaluated.

The virus evidently passes from the blood into the salivary glands, where it is mixed with the salivary secretions and passed into the plant when the leafhoppers feed. The relatively small amount of virus obtained from salivary glands removed from the leafhopper shows that these glands are not the chief virus reservoir. Other evidence suggests that the salivary glands may be somewhat unfavorable to the virus. It may be that the failure of leafhoppers to produce a normal amount of infection in the first and second 6-minute feedings after a long fasting period is due to partial inactivation of virus in the salivary glands by long contact with salivary secretions.

A more thorough study of the relation of the curly top virus to different tissues and fluids of nonvector species of insects, such as *Aceratagallia californica* and *Myzus persicae*, that pick up large quantities of virus and retain it for considerable periods, should shed light on the question of insect specificity in virus transmission. Since the virus retained its activity for several days in the bodies of the two species of insects named above and since both fed in the phloem, their inability to cause infection was not due to lack of active virus or to the tissue on which they fed, but evidently resulted from the presence of an effective barrier to virus passage in some part of the insect.

SUMMARY AND CONCLUSIONS

Beet leafhoppers, *Eutettix tenellus* (Baker), picked up virus from diseased beet plants in a feeding time of 1 minute, and viruliferous leafhoppers infected healthy plants in a feeding time of 1 minute. However, the minimum time for an individual leafhopper to pick up virus from a diseased plant and transmit it to a healthy plant was 4 hours.

Nonviruliferous leafhoppers placed on diseased plants acquired sufficient virus in 2 days to give them their maximum ability to infect

47419—38——4

seedling beets. A longer feeding period was necessary to enable them to acquire their maximum charge of virus.

A fasting period of 18 hours decreased the ability of leafhoppers to produce infection in the first two 6-minute feedings following the fast period. Feedings following a fast period of 15 minutes to 3 hours produced more infection than those following fast periods of 1 to 6 minutes or those following fast periods longer than 3 hours.

Virus was obtained from the blood, salivary glands, feces, and alimentary tract of the leafhopper, indicating a rather general distribution of the virus in the insect. However, the more abundant recovery of virus from the blood indicates that the blood is the chief virus reservoir.

Both virus content and ability to cause infection gradually decreased over periods of 8 to 10 weeks in viruliferous leafhoppers when the leafhoppers were confined to a very resistant or immune plant (Australian saltbush or sweet corn). This was true regardless of the size of the initial charge of virus in the leafhoppers when placed on the resistant or immune plant.

Leafhoppers that had a low charge of virus, acquired by a 6-hour feeding on an extract from viruliferous leafhoppers, lost the power to cause infection after 54 days when transferred daily on small beet plants. However, leafhoppers that had lost the power to cause infection regained it when allowed a short feeding period on curly top beets. These facts prove conclusively that if there is any multiplication of the virus in the leafhopper, it is not sufficient to maintain the original virus content. It seems probable that there is no multiplication of virus in the leafhopper.

Individual leafhoppers vary greatly in their ability to transmit virus. The results of selecting and mating leafhoppers in successive generations indicated that it is possible to produce strains lower than normal and higher than normal in ability to transmit virus to seedling beets in short-interval feedings. However, no individuals inherently lacking in ability to transmit virus were discovered and the results indicate that if such individuals exist within the species they are rare.

The following nonvector species of insects acquired virus by feeding on curly top plants: *Myzus persicae*, *Aphis rumicis*, *Pemphigus betae*, *Aceratagallia californica*, *Phlepsius strobi*, *Empoasca solana*, *Hercothrips femoralis*, and *Tetranychus* sp. When transferred to healthy plants, these insects retained the virus for periods ranging from less than 1 day, in the case of *H. femoralis*, to 21 days, in the case of *A. californica*.

LITERATURE CITED

(1) Ball, E. D.
1906. The beet leaf-hopper (Eutettix tenella). Utah Agr. Expt. Sta. Ann. Rept. 16: 16.

(2) Bennett, C. W.
1935. Studies on properties of the curly top virus. Jour. Agr. Research 50: 211–241, illus.

(3) Boncquet, P. A., and Hartung, W. J.
1915. The comparative effect upon sugar beets of Eutettix tenella Baker from wild plants and from curly top beets. (Phytopath. Note) Phytopathology 5: 348–349, illus.

(4) Carsner, E., and Stahl, C. F.
1924. Studies on curly-top disease of the sugar beet. Jour. Agr. Research 28: 297–320, illus.

(5) FISHER, R. A.
1934. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London.
(6) FREITAG, J. H.
1936. NEGATIVE EVIDENCE ON MULTIPLICATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, EUTETTIX TENELLUS. Hilgardia 10: 305–342, illus.
(7) FUKUSHI, T.
1934. STUDIES ON THE DWARF DISEASE OF RICE PLANT. Jour. Faculty Agr., Hokkaido Imp. Univ. 37: 41–164, illus.
(8) ———
1935. MULTIPLICATION OF VIRUS IN ITS INSECT VECTOR. Imp. Acad. Tokyo Proc. 11: 301–303.
(9) KUNKEL, L. O.
1926. STUDIES ON ASTER YELLOWS. Amer. Jour. Bot. 13: 646–705, illus.
(10) SEVERIN, H. H. P.
1921. MINIMUM INCUBATION PERIODS OF CAUSATIVE AGENT OF CURLY LEAF IN BEET LEAFHOPPER AND SUGAR BEET. Phytopathology 11: [424]–429, illus.
(11) ———
1924. CURLY LEAF TRANSMISSION EXPERIMENTS. Phytopathology 14: [80]–93, illus.
(12) ———
1931. MODES OF CURLY-TOP TRANSMISSION BY THE BEET LEAFHOPPER, EUTETTIX TENELLUS (BAKER). Hilgardia 6: [253]–276, illus.
(13) ———
1934. WEED HOST RANGE AND OVERWINTERING OF CURLY-TOP VIRUS. Hilgardia 8: 263–280, illus.
(14) ——— and FREITAG, J. H.
1933. SOME PROPERTIES OF THE CURLY-TOP VIRUS. Hilgardia 8: 1–48, illus.
(15) ——— and HENDERSON, C. F.
1928. SOME HOST PLANTS OF CURLY TOP. Hilgardia 3: 339–392, illus.
(16) SHAW, H. B.
1910. THE CURLY-TOP OF BEETS. U. S. Bur. Plant Indus. Bull. 181, 46 pp., illus.
(17) SMITH, R. E., and BONCQUET, A.
1915. NEW LIGHT ON CURLY TOP OF THE SUGAR BEET. Phytopathology 5: [103]–107, illus.
(18) STAHL, C. F., and CARSNER, E.
1918. OBTAINING BEET LEAFHOPPERS NONVIRULENT AS TO CURLY-TOP. [Preliminary paper.] Jour. Agr. Research 14: 393–394.
(19) STOREY, H. H.
1931. THE INHERITANCE BY A LEAFHOPPER OF THE ABILITY TO TRANSMIT A PLANT VIRUS. Nature [London] 127: 928.
(20) ———
1933. INVESTIGATIONS OF THE MECHANISM OF THE TRANSMISSION OF PLANT VIRUSES BY INSECT VECTORS.—I. Roy. Soc. [London], Proc., Ser. B, 113: 463–485, illus.

INFLUENCE OF CERTAIN HARMFUL SOIL CONSTITUENTS ON SEVERITY OF PYTHIUM ROOT ROT OF SUGARCANE [1]

By R. D. RANDS, *senior pathologist*, and ERNEST DOPP, *assistant pathologist*, *Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

The severity of root rot of the very susceptible noble varieties of sugarcane (*Saccharum officinarum* L.), and particularly of the more tolerant and now generally planted hybrid seedlings in Louisiana, depends partly at least upon the intensity of various host-conditioning factors. The principal root-rotting fungus, *Pythium arrhenomanes* Drechsler, may be readily isolated from practically every field and from the root systems of both good and poor cane. Its dispersion through the soil is favored by the usually abundant rainfall apparently whenever conditions, of either transitory or permanent nature, permit abundant root infections and rapid decay.

A high water table combined with fine texture of soil and low winter and spring temperatures are probably the chief indirect causes of severe outbreaks of root rot on present commercial varieties. Poor drainage in the Louisiana sugar belt is characteristic of the extensive low-lying areas of Sharkey clay and many of the transitional "mixed lands" separating the former from the higher lying sandy loams bordering the Mississippi River and numerous bayous. These heavy clays and clay loams have been variously estimated to occupy from 20 to 40 percent of the average sugarcane farm. With frequent and excessive rainfall they may remain practically saturated for periods of a week or longer. This would favor the possible formation of substances in the soil inimical to cane growth. Some reduction of both inorganic and organic soil compounds might be expected, with at least the formation of subtoxic concentrations of some of the reduced products. Should these exert a predisposing effect on the roots for pythium attack, the unusually severe root rot, arrested growth, yellowing of foliage during periods of drought, and delayed stooling suffered during some seasons by ordinarily root rot-tolerant varieties might be explained.

During the course of a detailed investigation of the relation of environmental factors to the severity of root rot the possible predisposing effect of various deleterious substances that may arise under conditions of poor soil aeration and drainage was studied. This paper presents the results of such experiments with hydrogen sulphide and salicylic aldehyde.

[1] Received for publication July 2, 1937; issued March 1938.

[2] The writers are indebted to P. R. Dawson, senior biochemist, Division of Soil Fertility Investigations, Bureau of Plant Industry, for critical reading of the manuscript.

REVIEW OF LITERATURE

No soils investigations have been conducted in relation to this specific problem in Louisiana, although Flor (*1*)[3] attempted to determine whether the severe root rot in seepage areas along the Mississippi River might not be due to the accumulation of toxins. His failure to demonstrate toxins might possibly have been due to oxidative and other beneficial changes taking place in the untreated seepage soil during the considerable period between its collection and use in his greenhouse experiments.

In studies of the decomposition of cane trash in Louisiana sugarcane soils, Sturgis (*13*) found that intermittent high moisture during late winter and spring greatly retarded decomposition and nitrification and appeared to bring on a toxic condition which disappeared relatively slowly when better drainage and higher temperature prevailed. More recently Sturgis (*14*) has reported on the effect of waterlogging and organic matter on the rate, intensity, and amount of reduction in Sharkey soils incident to the growing of rice. Addition of organic matter markedly increased the intensity of reduction and accumulation of reduced iron and manganese. Robinson (*4*) found unusually large amounts of soluble iron and manganese and the presence of sulphides in poorly drained spots in fields where corn grew poorly.

With respect to the occurrence of harmful organic compounds in soils, the earlier extensive investigations of Schreiner, Shorey, Skinner, and associates (*5*, *6*, *7*, *8*, *9*, *10*, *11*) may be cited. They isolated a whole series of injurious compounds from unproductive soils characterized by poor aeration, low biologic activity, deficient or poor soil preparation, and excessive moisture content. Deficiency of oxygen in the decomposition of plant residues caused the accumulation of such intermediate and injurious products as vanillin, salicylic aldehyde, acrylic acid, dihydroxystearic acid, and benzoic acid, all of which were injurious to plant growth in relatively low concentrations in water cultures.

MATERIALS AND METHODS

A standardized method for growing sugarcane in sand-nutrient cultures and the writers' root rot inoculation procedure have been partly described in an earlier publication (*2*). The method consists essentially in the use of a high-purity Pennsylvania quartz sand in 3-gallon glazed crocks provided with a ½-inch drain hole in the bottom from which a rubber tube conducts excess liquid to a 2-quart mason jar placed below each crock (fig. 1). The jar is painted black to prevent the growth of algae. It holds approximately 1,900 cc, and the sand in the crock, depending upon its fineness, retains against gravity about 2,000 to 2,600 cc, making a total of about 4 to 4.5 liters in the system. Daily, or twice daily, between changes of nutrient, the contents of the jar are poured over the sand in the crock, and any deficiency of leachings noticeable in the jar from evaporation and transpiration during the preceding day is made up by the addition of distilled water. This procedure not only simulates the probable effect of rain in dispersal of and infection by the fungus, but tends to equalize concentration of the solution throughout the culture.

[3] Reference is made by number (italic) to Literature Cited, p. 67.

The nutrient solution is partly or completely renewed at weekly or longer intervals, according to the requirements of the plants. Hydrogen-ion determinations on fresh leachings are made weekly or more frequently, and the ratio of ammonia to nitrate ions is so varied as to maintain a slightly acid reaction, about pH 6.0, which is favorable

FIGURE 1.—Method of growing sugarcane in sand-nutrient cultures. A rubber drain tube from the bottom of each crock conducts excess nutrient into the jars, the contents of which are periodically made up to volume and poured back into the crocks to equalize the concentration throughout the culture.

for cane growth. The nutrient solution D, used in most of the writers' experiments, contains the following salts in the partial volume-molecular concentrations indicated: NH_4NO_3, 0.00225; $Ca(NO_3)_24H_2O$, 0.00225; KH_2PO_4, 0.0015; K_2HPO_4, 0.0005; $MgSO_4$, 0.001; KCl, 0.0025; plus rarer elements in parts per million, Fe 5.0, Mn 1.0, B 0.5, Al 0.5, Cu 0.25, Zn 0.25, Br 0.25, and traces of Si and Ti.

Young sugarcane plants of uniform height and stature, previously sprouted in 4-inch pots of sand, are reset in the crocks with their attached 2- to 3-inch cutting and without disturbing the roots. After a week to 10 days the plants have become established and are supported at least partly by the development of their permanent or shoot root system. At this time treatments are applied and the various replicates of the treatments are placed in randomized arrangement on the greenhouse benches. After another week the cultures that are to receive the root-rotting pythium are inoculated.

Since an important effect of root rot is a retardation of growth and reduction in the number of suckers, or tillers, each experiment was continued for 2 to 3 months, or until further growth represented merely elongation of existing shoots. This was followed by weekly measurements of height of all shoots per crock. Harvesting after 2 to 3 months gave practically the same percentage difference between objects as after 7 months or until nearly full-grown stalks had developed.

HYDROGEN SULPHIDE

Hydrogen sulphide at initial concentrations of 10 and 50 p. p. m. in nutrient D was studied for effect on growth and root rot of the very susceptible D–74 variety grown in quartz sand cultures. The cuttings were sprouted and a uniform group of plants established in 3-gallon crocks, with two plants per crock. The hydrogen sulphide gas produced from iron sulphide in a Kipp generator was first bubbled through a water trap to remove any accompanying hydrochloric acid and then conducted into a measured volume of distilled water until the water was saturated. The temperature of the saturated solution and published solubility data permitted calculation of the volume of solution required to be added to each lot of dilute nutrient to give hydrogen sulphide concentrations of 10 and 50 p. p. m., respectively. The rarer elements ordinarily added to the nutrient were withheld because the iron, copper, and zinc salts would be precipitated by the hydrogen sulphide. They were added the following day to the receiving jars before the contents of the jars were poured over the crocks.

Inoculations were made May 5, 1932, with the authors' mildly parasitic strain No. 730 of *Pythium arrhenomanes*. The treatments were started a week later. Each treated series was divided into six inoculated and six control crocks, and a similar untreated series served for comparison. Fresh treatments were applied weekly. Under the well-aerated condition of these cultures, the hydrogen sulphide was evidently rapidly oxidized, since no odor was detectable in displaced nutrient that had occupied the sand for 24 hours. This was accompanied by a slightly greater increase of acidity in the nutrient of the 50-p. p. m. treatment.

Twice weekly and, for a period, daily determinations of pH revealed a rapid increase in acidity in all series from the initial reaction of pH 6.0 for fresh nutrient to as high as pH 3.8 and 4.0 at the end of the first month. This was due to the use of ammonium nitrate as the sole source of nitrogen in nutrient D. Thereafter part of the total nitrogen was added as calcium nitrate, and the ratio of the two salts was varied to maintain the reaction between pH 5.2 and 6.5.

The experiment extended from May 5 to July 26, 1932. The plants in the untreated and uninoculated series particularly showed excellent development, and those in the remaining series, while much retarded and deficient in suckering, showed at no time any unhealthy appearance of foliage. Mean total height of plants and dry weight of tops per crock are given in table 1.

TABLE 1.—*Effect of hydrogen sulphide on growth and root rot of the very susceptible D–74 variety of sugarcane*

Treatment	Total length of shoots			Dry weight of tops		
	Mean per crock	Reduction due to—		Mean per crock [1]	Reduction due to—	
		H_2S	Fungus		H_2S	Fungus
	Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
None:						
Controls	158			95.9		
Inoculated	86		46	66.3		30.9
H_2S, 10 p. p. m.:						
Controls	128	19		75.6	21.2	
Inoculated	79		38	52.6		30.4
H_2S, 50 p. p. m.:						
Controls	125	21		70.7	26.3	
Inoculated	70		44	45.2		36.1

[1] Difference between means required for odds of 19 : 1=9.8 g, and for odds of 99 : 1, 13.1 g.

Mean plant weights and calculations in table 1 indicate severe damage from the sulphide treatments in the absence of the fungus. This was expected in view of the well-known poisonous action of soluble sulphides on most crops. The noticeably darkened appearance of the sand receiving the 50 p. p. m. of hydrogen sulphide indicated extensive precipitation of insoluble sulphides on the particles, but the roots remained white and were merely deficient in length and branching.

Any weakening or unhealthy effect of the hydrogen sulphide apparently did not cause significantly greater damage by the fungus, as the last column of table 1 shows. In fact, the yield reductions caused by the fungus are not significantly different in any of the series, which suggests that in the concentrations employed hydrogen sulphide was without influence on susceptibility of the plants to root rot.

SALICYLIC ALDEHYDE

Among the many harmful organic compounds isolated by Schreiner, Shorey, Skinner, and associates from unproductive soils were several of aldehyde nature (*8*) that proved to be common constituents of the soil organic matter. Salicylic aldehyde, vanillin, and trithiobenzaldehyde were definitely identified and studied in relation to crop growth. They were considered to have been either directly introduced in certain plant remains or formed as intermediate reduction products during the decomposition of vegetable residues under conditions of high acidity or in soils low in oxidizing power and biological activity.

Salicylic aldehyde was identified by Shorey (*10*), and greenhouse and field tests showing its injurious effect on plant growth were reported by Schreiner and Skinner (*8*) in 1914. More comprehensive

studies on the occurrence and persistence of aldehydes in garden and field soils and their influence on crop production were summarized by Skinner (*11*) in 1918. Ten parts per million of salicylic aldehyde in nutrient solution cultures reduced the growth of wheat by 27 percent. Generally, less damage resulted from additions to soils; in fact, no detrimental effect whatever was determined for certain fertile soils well supplied with lime and phosphates.

Since the occurrence and persistence of aldehydes in soils were often associated with poor drainage and low or temporarily interrupted biological activity—conditions which characterize large areas in late winter and spring in the Louisiana sugar district—it seemed advisable to determine the possible influence of aldehydes on growth and root rot of sugarcane. However, as previously mentioned, it has not been demonstrated that such substances actually occur in Louisiana soils.

Preliminary greenhouse tests with commercial preparations of both salicylic aldehyde and vanillin suggested greater tolerance of sugarcane to these compounds than Schreiner and Skinner observed in several other crops. However, these differences may be attributable to differences in the nutrient solutions and technique employed. Therefore, a commercial preparation of salicylic aldehyde, selected for ease of handling, was tested in a series of more adequately replicated greenhouse experiments.

First, it was necessary to ascertain what effect this compound would have on the root-rotting pythium alone. Some idea of this was gained by comparing growth of the fungus on agar containing different concentrations of the chemical. Concentrations of 10, 25, and 50 p. p. m. were made up in melted and previously sterilized corn-meal agar in flasks, and a series of Petri dishes was poured with each, besides which there was an untreated series. On cooling, the dishes were inoculated at one side with a pure culture of *Pythium arrhenomanes*. Average rate of growth after 24 hours revealed no significant differences between the control and the 10 and 25 p. p. m. series; in the 50 p. p. m. no growth whatever had taken place, and subsequent examination showed that the fungus had been killed in all plates of this series. In the lower concentrations, therefore, salicylic aldehyde was without visible effect on the appearance and rate of growth of the pythium in culture.

Next, preliminary qualitative tests were conducted to determine the persistence of salicylic aldehyde in 3-gallon crocks of sand without plants but provided with drain tubes and receiving jars as in the experiments. Most of the liquid in the sand of two crocks was replaced by additions of nutrient containing 25 and 50 p. p. m., respectively, of the aldehyde. At daily intervals the solution was displaced into the receiving jars and aliquots were removed and tested against standards with ferric chloride. The contents of the receiving jars that contained the aldehyde were then poured back over the sand and similarly replaced and tested on succeeding days.

Seventy-two hours after the original application there was no longer any visible reaction for aldehydes with ferric chloride in the case of the 25 p. p. m. dosage, and only a faint change in color was noted in the leachings of the 50 p. p. m. application. The latter was similar to that of a 5 p. p. m. control, which concentration is about the minimum detectable by the naked eye with this indicator. Therefore, within

3 days most of the salicylic aldehyde applied to this sand was apparently absorbed or oxidized.

EXPERIMENT 1

In experiment 1 an approximate concentration of 20 p. p. m. salicylic aldehyde in nutrient solution was tested in sand cultures of sugarcane in the presence and in the absence of the moderately virulent strain 58 of *Pythium arrhenomanes*. Comparable control series with nutrient alone with and without the fungus served for comparison of separate and combined effects of treatment and pythium.

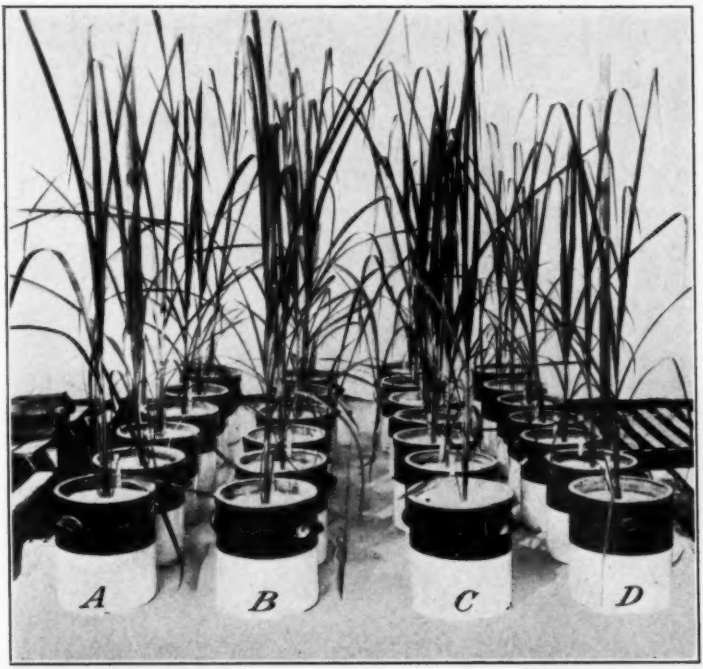

FIGURE 2.—Influence of salicylic aldehyde on susceptibility of sugarcane to root rot in sand-nutrient cultures: *A*, Control; *B*, aldehyde alone; *C*, pythium alone; *D*, combination of aldehyde and pythium.

Thirty-three glazed crocks of 3-gallon capacity were filled with new quartz sand, wetted with tap water, autoclaved for 2 hours at 15 pounds' steam pressure, cooled, and leached with 2 liters of distilled water each. The crocks were provided with a central drain hole in the bottom, but in this test neither drain tubes nor receiving jars were employed. On December 8, 1931, a uniform series of young plants of the moderately susceptible Co. 281 variety growing in 4-inch pots of similarly steamed sand were transplanted, one per crock, and given 1 liter of distilled water. On December 10, inoculations with strain 58 of *Pythium arrhenomanes* were made to the fungus series and similar but sterile media were added to the controls. On December 14, 1

liter of nutrient solution containing 50 p. p. m. salicylic aldehyde was added to the aldehyde series, and nutrient alone to the controls. Uncertain, and at the time inadequate, supplies of distilled water prohibited the addition of exactly 20 p. p. m. of the aldehyde in a volume of nutrient sufficient to replace at one time all liquid (approximately

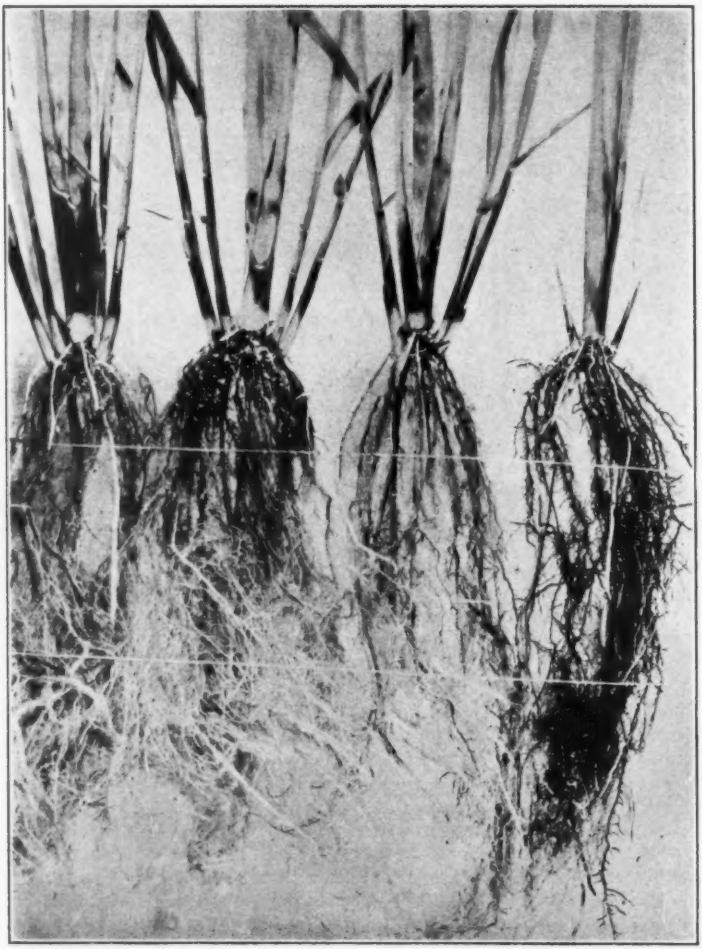

FIGURE 3.—Comparative extent of suckering (tillering) and condition of root systems of representative plants in experiment 1. Left to right: Control, aldehyde alone, pythium alone, and aldehyde plus pythium.

2,500 cc) retained against gravity by this sand. However, the 50 p. p. m. salicylic aldehyde in 1,000 cc of nutrient actually applied is assumed to have diffused to some extent through the approximately 1,500 cc of liquid not displaced from the sand and was thereby diluted to about 20 p. p. m. of total liquid. Tests indicated that some diffusion

at least had taken place soon after application, because the addition of another liter of nutrient or distilled water gave leachings with sufficient aldehyde for detection with ferric chloride. Furthermore, growth measurements of the plants showed that the fungus was active, whereas in the aforementioned test in agar cultures it had been killed in a concentration of 50 p. p. m. Nevertheless, it was unfortunate that a more uniform initial concentration of the chemical could not be placed in contact with the root systems.

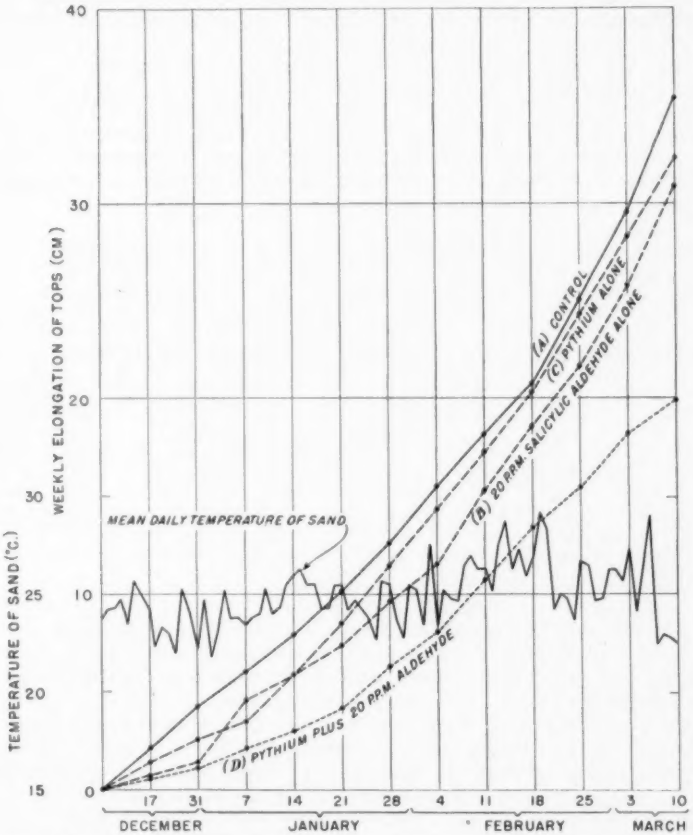

FIGURE 4.—Mean daily temperature of sand, and weekly elongation of tops *A–D*, reflecting damage from root rot in the presence and in the absence of salicylic aldehyde in sand cultures.

The experiment extended from December 10, 1931, to March 10, 1932. Growth was normal in appearance and extent for this season of the year, with gradual acceleration in rate of elongation as temperatures rose and the period of sunshine increased toward the end of the test. Plants receiving both the treatment and fungus (series D) often showed temporary wilting at midday, which indicated a deficiency of absorbing root area. Figure 2, from a photograph taken

nearly 3 weeks prior to the termination of the experiment, illustrates the smaller size in series D and the absence of noticeable differences between the remaining series.

Striking damage from the combination of aldehyde and fungus was revealed on examination of the root systems, as is shown by the representative comparison in figure 3. Whereas the fungus alone caused extensive rootlet tip damage, in the presence of the aldehyde it rotted completely most of the finer roots and invaded and suppressed many of the larger ones. The greatly reduced absorbing root area that resulted was reflected in a slow elongation of the primary shoot and an absence of normal suckering (tillering).

Mean height and green weight of plants are presented in table 2 and shown graphically in figure 4. There was no significant difference between the controls and the aldehyde-treated plants in the absence of the fungus. The fungus alone caused the usual significant reduction for greenhouse tests with this variety, but in the presence of the aldehyde the damage was more than twice as great, which suggests a predisposing effect of the aldehyde for root rot.

TABLE 2.—*Effect of salicylic aldehyde on growth of sugarcane variety Co. 281 and the severity of root rot caused by Pythium arrhenomanes in experiment 1*

Series	Treatment	Plants	Mean height	Difference due to—		Mean green weight of tops [1]	Difference due to—	
				Aldehyde	Pythium		Aldehyde	Pythium
		Number	*Centimeters*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
A	None (controls)	6	35			180.3		
B	Aldehyde alone, 20 p. p. m	7	31	−11		185.1	+2.7	
C	Pythium alone	10	32		−8	141.3		−21.6
D	Pythium plus 20 p. p. m. aldehyde	10	20		−35	90.3		−51.2

[1] Difference between means required for odds of 19 : 1=26.7 g., or 14.8 percent of control (A) series.

EXPERIMENT 2

Experiment 1 was repeated with the following modifications as experiment 2: Weekly applications of 50 p. p. m. of salicylic aldehyde on the basis of 2 liters of nutrient D were made to sand cultures of Louisiana Purple, a very susceptible variety, and to C. P. 807, a variety highly resistant to root rot, both in the presence and in the absence of the mildly parasitic strain No. 730 of *Pythium arrhenomanes*. Large galvanized cans, 14 inches in diameter by 24 inches high, coated inside with paraffin and holding 100 pounds of sand, were used in place of crocks. The cans were provided with drain outlets and receiving jars. Four plants were set in each can, and there were five cans per object, two with Louisiana Purple and three with C. P. 807.

The test plants were set in the cans on March 24 and inoculated March 25, and the first aldehyde application was made on March 28, 1932. Five additional applications in 2 liters of nutrient were made at weekly intervals until May 10, when the plants were harvested. Weekly pH determinations on leachings gave values between pH 5.4 and 6.2. Daily the receiving jars were poured over the sand and

sufficient distilled water was added to maintain a fairly uniform moisture content. This procedure provided a 24-hour exposure of most of the roots to each full-strength (50 p. p. m.) aldehyde application once a week, which was followed by rapid diminution in concentration of the aldehyde until the next application.

All plants of both varieties made normal appearing growth and, except for the Louisiana Purple that received the combination of aldehyde and pythium, the plants tillered profusely. The average green weights per individual plant, shown in table 3, reveal for the susceptible Louisiana Purple variety a significantly predisposing effect from the aldehyde similar to that obtained in the preceding experiment (table 2). However, the root-rot-resistant C. P. 807 variety shows no significant reduction from either the combination or the pythium alone.

TABLE 3.—*Influence of salicylic aldehyde on growth and severity of root rot of susceptible and resistant varieties of sugarcane, experiment 2*

Variety and treatment	Height of primary shoots	Difference due to—		Mean green weight of tops [1]	Difference due to—	
		Aldehyde	Pythium		Aldehyde	Pythium
	Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
Louisiana Purple (susceptible to root rot):						
Untreated controls	21			166.1		
Aldehyde alone, 50 p. p. m	18	−14		144.9	−12.8	
Pythium alone	16		−24	141.7		−14.7
Pythium plus 50 p. p. m. aldehyde	14		−22	99.6		−31.3
C. P. 807 (resistant to root rot):						
Untreated controls	51			183.3		
Aldehyde alone, 50 p. p. m	40	−22		143.8	−21.5	
Pythium alone	48		−6	165.2		−9.9
Pythium plus 50 p. p. m. aldehyde	40		0	137.0		−4.7

[1] Means of individual plants with suckers; differences required for odds of 19:1 are, for Louisiana Purple, 28.7 g, or 17.3 percent of controls; and for C. P. 807, 33.7 g, or 18.4 percent of controls.

EXPERIMENT 3

Since an adequate supply of moisture was difficult to maintain in the upper portion of the deep cans employed in the preceding test, experiment 1 was repeated to test further the apparent predisposing effect of salicylic aldehyde on susceptibility to root rot. Six crocks with two plants each were used for comparing two concentrations of the aldehyde in the presence and in the absence of the fungus. The variety Co. 281 and the same strain (No. 58) of the fungus were again employed, but drain tubes and receiving jars were used to maintain a more uniform concentration of nutrient solution throughout the sand. Nutrient solution D was applied 2 liters per crock every other week, which provided ample nutrition for the plants.

Salicylic aldehyde was added to the 2 liters of nutrient solution at each biweekly partial renewal to make initial concentrations of 40 and 80 p. p. m., respectively. These were subsequently reduced by diffusion and some percolation into the receiving jars. Daily the contents of the jars were poured over the sand after being brought up to capacity with distilled water. Since they also held approximately 2 liters, the result was substantially an alternate day exposure of the roots to the aldehyde until it became oxidized or adsorbed by the

sand, i. e., after 4 to 6 days. Applications of nutrient and aldehyde were made on September 12, 4 days after planting, and repeated September 26, October 10 and 24, November 7 and 21, and December

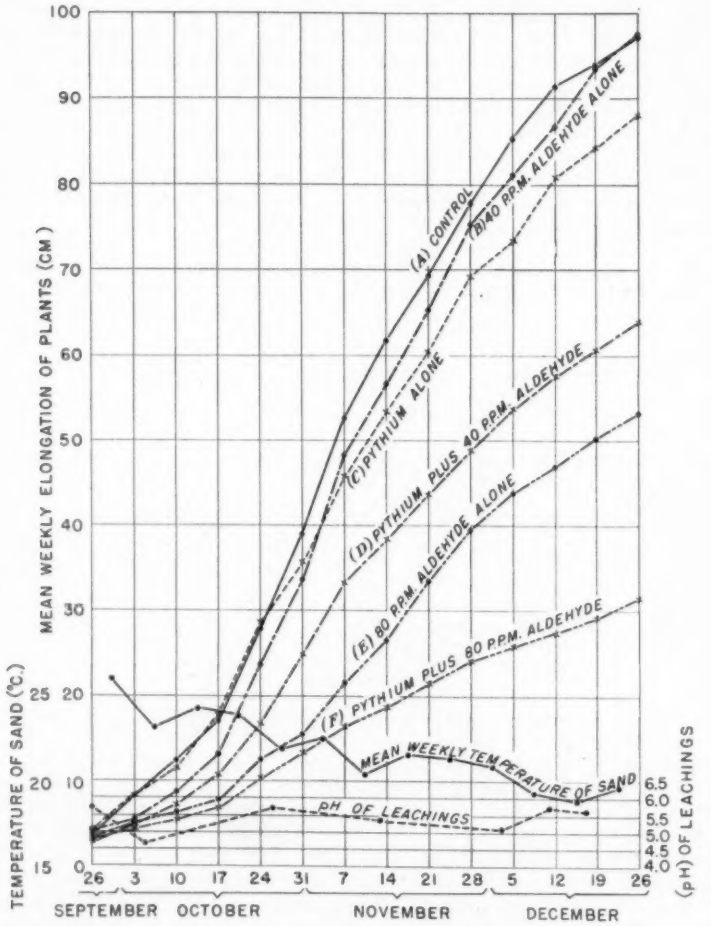

FIGURE 5.—Average weekly temperature of sand, pH of leachings, and growth curves *A–F* of plants grown in two concentrations of salicylic aldehyde in the presence and in the absence of the root-rotting fungus *Pythium arrhenomanes*.

5, 1934, making a total of seven partial renewals. Inoculations were made on September 14, and the test plants were harvested on December 28, 1934. The results are presented in table 4 and figure 5.

TABLE 4.—*Effect of biweekly applications of salicylic aldehyde on growth and severity of root rot of the moderately susceptible sugarcane variety Co. 281, experiment 3*

Series	Treatment (6 replications)	Mean total height per crock	Difference due to—		Mean green weight of tops [1]	Reduction due to—	
			Aldehyde	Pythium		Aldehyde	Pythium
		Centimeters	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
A	None (controls)	195			588.4		
B	Aldehyde alone, 40 p. p. m	196	+0.5		564.4	4.1	
C	Pythium alone	177		−9	556.0		5.5
D	Pythium plus 40 p. p. m. aldehyde	128		−35	389.2		31.0
E	Aldehyde alone, 80 p. p. m	107	−45		323.2	45.4	
F	Pythium plus 80 p. p. m. aldehyde	63		−41	204.6		36.7

[1] Differences between means required for odds of 19 : 1=83.6 g, and for 99 : 1=113.8 g.

The results of this experiment confirm those of experiment 1 (table 2) in demonstrating a significantly aggravating effect of salicylic aldehyde on root rot. Whereas alone neither the lesser concentration of the aldehyde nor the fungus caused appreciable damage, when the two were combined the plants were severely stunted. The growth curves in figure 5 suggest that the pythium was also more active at higher temperatures in the presence of the aldeyhde than in its absence. When the aldehyde was absent there was pronounced retardation of growth at temperatures below that required for vigorous cane growth (about 20° C.).

Applications of 80 p. p. m. of the aldehyde caused severe growth retardation without the aid of the fungus. Preliminary tests had shown that weekly dosages of 100 p. p. m. would cause severe wilting and eventual death of the plants, but the less frequent applications of 80 p. p. m. in this test resulted in no such abnormal external symptoms in the absence of the fungus. The roots were merely deficient in length and extent of branching and to all outward appearances were not different from untreated plants of the same size but much younger age. The pythium, no doubt, was also affected by the strong concentration of aldehyde, although with the gradual weakening or disappearance of the latter during the long intervals between successive applications, it was apparently still able to cause about seven times as much growth reduction as it had in the absence of aldehyde.

DISCUSSION

The significant increase in severity of root rot of sugarcane in the presence of salicylic aldehyde in these experiments is interpreted as the result of a conditioning or predisposing effect of the chemical on the roots which enabled the fungus to cause greater damage. The possibility that it might be due to a stimulating or nutritional effect on the fungus would appear to be excluded by the negative results from tests of the fungus in pure culture.

The results of these experiments suggest the desirability of soil investigations to explain certain anomalies connected with the occurrence of the disease in the field. Should this or similarly behaving

compounds be found in the root rot soils, possible corrective measures, such as improved drainage, special fertilization, and soil amendments, could be more intelligently instituted. The effect of increasing biological activity, improving aeration, moisture control, and possibly chemical conditions of such soils, on root health as well as on possible formation and accumulation of injurious or root rot-accentuating substances should be determined. The results of experiments by Robbins and Massey (*3*) suggested that the aldehyde vanillin is destroyed in the soil by certain micro-organisms, and Skinner and Noll (*12*) showed that the injurious effect on plants from its persistence in an unproductive, acid soil could be overcome by the addition of lime or of lime and phosphate. Possible insufficiency of these materials is not believed to have been a factor in the tests with salicylic aldehyde reported in this paper, because of the excellent growth of treated, uninoculated plants. Each 2-liter weekly or biweekly addition of nutrient solution D provided the equivalent of 25.9 pounds of calcium oxide and 29.3 pounds of phosphorus pentoxide per acre.

The cultivation of vigorous, root-rot-resisting varieties of sugarcane, such as Co. 290, C. P. 28/11, and C. P. 29/116, on the poorly drained, heavy clay soils of the Louisiana sugar district has assured, for the present at least, economical utilization of such areas. However, the question naturally arises: Should varieties alone without accompanying soil improvement be depended upon as a permanent or even as the most economical solution of production problems on such soils? A tentative answer is found in the renewed efforts of many growers to improve drainage by deepening field ditches and outlet canals and also to prevent caking, and at the same time to raise the general level of fertility by plowing under legumes and cane trash and occasionally by applications of factory filter-press cake and barnyard manure. Under such treatment the root-rot-resistant varieties have shown a marked response, and in a few instances the improvement has been so great that excellent yields of the rather susceptible Co. 281 have been obtained.

SUMMARY

During an investigation of host-conditioning factors that influence the severity of root rot caused by *Pythium arrhenomanes*, in the poorly drained, heavy clay soils of the Louisiana sugar district, the possible predisposing effect of deleterious substances, which might arise during temporary water-logging, was determined. Hydrogen sulphide and salicylic aldehyde were selected as examples and tested in greenhouse inoculation experiments with sugarcane growing in sand-nutrient cultures.

Hydrogen sulphide alone at concentrations of 10 and 50 p. p. m. in nutrient solution reduced the growth of tops approximately 21 and 26 percent, respectively; the pythium alone, 31 percent, and in combination with the sulphide, 30 and 36 percent, which latter percentages are not sufficiently different to indicate any special influence of the sulphide on disease intensity.

Salicylic aldehyde significantly increased susceptibility to root rot. Concentrations of 20 to 40 p. p. m., which had little if any influence on cane growth in the absence of the fungus and showed no effect on

the fungus in culture, apparently so predisposed the roots to infection that when the fungus was present the reduction in weight of plants was from two to seven times greater than from the fungus alone.

The presence of this or similarly behaving compounds in the poorly drained, heavy clay soils of the Louisiana sugar district would account at least partly for the frequently severe root rot noted in all but the most resistant varieties. Improving the drainage and the general level of fertility in such areas has in numerous instances markedly increased yields of even the resistant varieties.

LITERATURE CITED

(1) Flor, H. H.
1930. factors influencing the severity of the root rot troubles of sugar cane. La. Agr. Expt. Sta. Bull. 212, 40 pp., illus.

(2) Rands, R. D., and Dopp, E.
1934. variability in pythium arrhenomanes in relation to root rot of sugarcane and corn. Jour. Agr. Research 49: 189–221, illus.

(3) Robbins, W. J., and Massey, A. B.
1920. the effect of certain environmental conditions on the rate of destruction of vanillin by a soil bacterium. Soil Sci. 10: 237–246, illus.

(4) Robinson, W. O.
1930. some chemical phases of submerged soil conditions. Soil Sci. 30: 197–217.

(5) Schreiner, O., Reed, H. S., and Skinner, J. J.
1907. certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agr., Bur. Soils Bull. 47, 52 pp., illus.

(6) ——— and Lathrop, E. C.
1911. dihydroxystearic acid in good and poor soils. Jour. Amer. Chem. Soc. 33: 1412–1417.

(7) ——— and Shorey, E. C.
1909. the isolation of harmful organic substances from soils. U. S. Dept. Agr., Bur. Soils Bull. 53, 53 pp., illus.

(8) ——— and Skinner, J. J.
1914. harmful effect of aldehydes in soils. U. S. Dept. Agr. Bull. 108, 26 pp., illus.

(9) Shorey, E. C.
1913. some organic soil constituents. U. S. Dept. Agr., Bur. Soils Bull. 88, 41 pp., illus.

(10) ———
1914. the presence of some benzene derivatives in soils. Jour. Agr. Research 1: 357–363.

(11) Skinner, J. J.
1918. soil aldehydes. a scientific study of a new class of soil constituents unfavorable to crops, their occurrence, properties, and elimination in practical agriculture. Jour. Franklin Institute 186: 165–186, 289–316, 449–480, 547–584, 723–741, illus.

(12) ——— and Noll, C. F.
1916. field tests of fertilizer action on soil aldehydes. Jour. Amer. Soc. Agron. 8: 273–298, illus.

(13) Sturgis, M. B.
1932. the effect of additions of nitrogen on the decomposition of sugar cane trash under field conditions. Jour. Amer. Soc. Agron. 24: 690–706, illus.

(14) ———
1936. changes in the oxidation-reduction equilibrium in soils as related to the physical properties of the soil and the growth of rice. La. Agr. Expt. Sta. Bull. 271, 37 pp.

THE VITAMIN A VALUE OF BLUE GRAMA RANGE GRASS AT DIFFERENT STAGES OF GROWTH[1]

By MARGARET CAMMACK SMITH, *nutrition chemist, Department of Nutrition*, and E. B. STANLEY, *head, Department of Animal Husbandry, Arizona Agricultural Experiment Station*

INTRODUCTION

Livestock in the range area of Arizona, depending as they do almost exclusively upon native vegetation for their feed supply, are obviously subjected to conditions which make for a widely variable supply of the several feed essentials. Although Woods and her coworkers [2] have reported many range forages to be excellent sources of vitamin A, Hart and Guilbert [3] found that vitamin A deficiency occurred in range cattle under natural conditions, the deficiency developing when the dry feed was unusually prolonged.

In Arizona the grama grasses are the most widely distributed and the most valuable of the perennial grasses used for grazing purposes. Blue grama (*Bouteloua gracilis*) is probably the most abundant of these grasses between the elevations of 4,000 and 8,000 feet and is particularly prevalent on the grassland ranges. It grows to a height of from 10 to 20 inches and develops a good growth of fine basal leaves which remain partly green during the winter months, the extent of greenness depending upon the moisture available. The annual growth of this important range forage is initiated by the summer rains in late July. The plant grows rapidly, matures quickly, and by the middle of October begins to dry up. Except for some greening of the basal leaves in the early spring it shows no further growth until the following summer rains. During the dormant stage, the remaining old-growth part of the plant is dry and comparatively unpalatable.

As blue grama grass constitutes one of the principal sources of vitamin A for range cattle in certain sections of Arizona, the authors have sought to ascertain the vitamin A potency of this nutrient for range cattle at different stages of its growth.

EXPERIMENTAL PROCEDURE

The samples of blue grama used in this study were collected from a grassland type of range in southern Arizona in the vicinity of Sonoita at an elevation of 4,000 feet. Three sets of samples were taken at different stages in the growth of the plant. The first collection was made the first week in August 1936, about 2 weeks after

[1] Received for publication July 19, 1937; issued March 1938.

[2] WOODS, E., ATKESON, F. W. SHAW, A. O., SLATER, I. W., and JOHNSON, R. F. THE VITAMIN A CONTENT OF PASTURE PLANTS. III. ALFALFA (MEDICAGO SATIVA L.) AND SMOOTH BROME (BROMUS INERMIS LEYSS.) UNDER PASTURAGE CONDITIONS AND FED GREEN. Jour. Dairy Sci. 18: 573–578, illus. 1935.

——— ATKESON, F. W., SLATER, I. W., ARNDT, C. D., and JOHNSON, R. F. VITAMIN A CONTENT OF PASTURE PLANTS. IV. WHITE BLOSSOM SWEET CLOVER (MELILOTUS ALBA DESVX.). ORCHARD GRASS (DACTYLIS GLOMERATA L.) AND MEADOW FESCUE (FESTUCA ELATOIR L.) UNDER PASTURAGE CONDITIONS AND FED GREEN. Jour. Dairy Sci. 18: 639–645. illus. 1935.

——— ATKESON, F. W., WELLHOUSEN, H., and JOHNSON, R. F. VITAMIN A CONTENT OF PASTURE PLANTS. II. TIMOTHY (PHLEUM PRATENSE L.) AND RED TOP (AGROSTIS ALBA L.) UNDER PASTURAGE CONDITIONS AND FED GREEN. Jour. Dairy Sci. 18: 547–556, illus. 1935.

——— SHAW, A. O., ATKESON, F. W., and JOHNSON, R. F. VITAMIN A CONTENT OF PASTURE PLANTS. I. WHITE CLOVER (TRIFOLIUM REPENS) AND KENTUCKY BLUE GRASS (POA PRATENSIS) UNDER PASTURAGE CONDITIONS AND FED GREEN. Jour. Dairy Sci. 15: 475–479, illus. 1932.

[3] HART, G. H., and GUILBERT, H. R. VITAMIN A DEFICIENCY AS RELATED TO REPRODUCTION IN RANGE CATTLE. Calif. Agr. Expt. Sta. Bull. 560, 30 pp., illus. 1933.

new growth had started; the second was made on September 20 when the grass was in the mature stage, and the third 2 months later, in November, when the plants were dormant and had undergone considerable weathering.

The samples were obtained by clipping the plant about 1½ inches above the ground. Only plants that had not been previously clipped or grazed were collected. The grass was placed in muslin bags and sent to the nutrition laboratory at the University of Arizona to be tested for vitamin A. As soon as it was received there it was dried rapidly by forced ventilation, ground, and all not needed for immediate use was transferred to covered glass jars, and kept in a refrigerator to minimize loss in vitamin content during storage. Tests were begun at once with albino rats which had been depleted of their vitamin A reserves. The method of Sherman and Munsell as modified by Sherman and Burtis [4] was used. It consisted in feeding the grass as the sole source of vitamin A in an otherwise adequate diet to rats whose vitamin A reserves had been exhausted by subsistence upon the same vitamin-A-free ration, and using the growth response as a measure of the concentration of vitamin A in the grass.

TABLE 1.—*Summarized results of feeding range blue grama grass, sampled in 3 consecutive months, to albino rats as the sole source of vitamin A, 1936*

Month of sampling	Amount of glue grama grass in ration	Rats used in test	Average food consumed per day	Average gain or loss in body weight per week	Average survival in test period
	Percent	*Number*	*Grams*	*Grams*	*Days*
August	0.00	6		(1)	32
	.025	4	5.6	(1)	43
	.05	10	8.1	6.5	
	.10	10	9.1	13.3	
	.50	10	9.2	18.0	
	1.00	7	12.3	23.0	
September	.00	4		(1)	
	.05	8	5.4	(1)	35
	.10	8	8.1	5.0	
	.20	8	11.4	10.7	
November	.00	5		(1)	28
	1.00	10	8.3	(1)	41
	2.50	[2] 8	8.8	2.0	(1)
	5.00	12	9.5	6.5	
	10.00	12	13.2	13.3	

[1] Loss.
[2] Three died on forty-fifth day.

In preliminary experiments weighed portions of the dry grass were fed separately. Although the rats readily ate small portions of the young green dry grass cut in August, they found the November grass less palatable and refused large portions of it. For this reason the grass was incorporated in the base ration at a definite percentage level in place of an equivalent percentage of cornstarch. The grass had to be ground very fine to make its separation from the other ingredients of the ration impossible, for there was a marked tendency on the part of the rats to separate out and leave the tougher grass. During the 8-week test period, the rats were housed in individual

[4] SHERMAN, H. C., and BURTIS, P. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680. 1938.

round metal cages with raised screen bottoms. They were allowed to eat ad libitum of the experimental diet (vitamin-A-free in which the grass was incorporated at various percentage levels), and tap water was before them at all times. Careful records of the food consumption and weight of each rat and observations on its general health were made weekly. Autopsies were made on all rats at death or termination of experiment, and all infected lesions and other signs of an inadequate intake of vitamin A were noted.

EXPERIMENTAL DATA

The results of the experiments, summarized in table 1, show that blue grama grass in its early-growth stage is an excellent source of vitamin A. Inclusion of as little as 0.05 percent of the dried grass (approximately 0.004 g daily as computed from food-consumption records) as the only source of vitamin A in a basal vitamin-A-free ration stimulated a rate of growth of approximately 6.5 g per week in the rats subsisting on this diet.

When the same grass cut a month later was tested for its vitamin A content twice as much was required to produce approximately the same rate of gain. The grass was bright green in color, but the blades were tougher and thicker. Blue grama grass at this stage can still be considered a rich source of vitamin A, comparing favorably with the leaves of highest grade alfalfa. By November, however, the blue grama grass had lost, along with its green color, much of its vitamin A. When the November grass was fed as the only source of vitamin A, 5 percent was required to induce an average rate of gain of 6.5 g weekly in the test animals, or 100 times the amount required of the young, tender August grass. Thus a striking deterioration is shown in the nutritive value of the grass in a rather short period of time.

From the foregoing results it appears that blue grama grass is extremely rich in vitamin A at certain stages of its growth, but for the greater part of the year it is relatively deficient in this vitamin. It would seem that range cattle, feeding upon the new growth, would be amply provided with vitamin A, and in fact have access to an amount sufficient to establish a reserve supply. They would, however, be dependent upon this reserve for the greater part of the year because of the striking early decrease in the vitamin A content of their food supply.

SUMMARY

Blue grama range grass at different stages of growth has been tested for vitamin A value by the rat-growth method. When cut in August, only 0.05 percent of the dry grass in a vitamin-A-free ration induced a gain of 6.5 g per week in the rats, thus showing the grass to be an extremely potent source of vitamin A at this stage of its development. Approximately twice as much of the grass cut in September and 100 times as much cut in November was necessary to produce the same rate of gain.

ADAPTATION AND USE OF AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT [1]

By H. D. CHAPMAN, *assistant chemist*, and GEORGE F. LIEBIG, JR., *associate in the experiment station, California Agricultural Experiment Station*

INTRODUCTION

For the control of nutrient solution concentration in sand cultures, the automatic periodic flushing system described by Eaton [2] possesses many advantages over the various drip-culture methods. The essential components of a type unit, as described by Eaton, consist of: (1) A large reservoir for the storage of the culture solution, (2) the sand culture, and (3) a motor-driven pump controlled by a time clock. In operation, the sand cultures are periodically flushed with the nutrient solution contained in the reservoirs, the excess solution from the cultures draining by gravity back into the reservoir. The capacity of the reservoir being large as compared with that of the sand cultures, concentrations may be maintained within closely prescribed limits by the addition of water and salts as needed and by occasional renewal.

For the motor-pump unit utilized by Eaton, the authors have substituted a simple glass ejector-type air lift operated by compressed air. By means of a clock-controlled magnetic valve inserted in the air line, the pumps are automatically started and stopped. With an appropriate compressor and storage tank, any number of units can be operated simultaneously. This simple and inexpensive modification is particularly adapted, though not necessarily limited, to greenhouse installations, where space is usually restricted. The present paper gives a brief description of an indoor (2-gallon sand culture) and an outdoor (25-gallon sand culture) set-up, and records certain experiences gained and results secured during the course of more than a year's work.

DESCRIPTION

The general features of the outdoor and indoor equipment are shown in figure 1, and further details of construction are given in figure 2.

AIR LIFT

The glass air lift is composed of three parts: An air inlet tube (*a*), the ejector tube (*b*), and a distributor (*c*), the last named being made from a 500-ml wide-mouthed bottle with the bottom cut off. In operation, air under pressure entering the open glass tube of the ejector member at point *d* lifts the solution into the distributor where it flows by gravity through glass distributor tubes to the sand cultures. Ejector tubes with an inside diameter of 0.5 inch have proved satisfactory for both the small and large units. The compressed-air tube

[1] Received for publication July 6, 1937; issued March 1938. Paper no. 373, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

[2] EATON, F. M. AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT. Jour. Agr. Research 53: 433–444, illus. 1936.

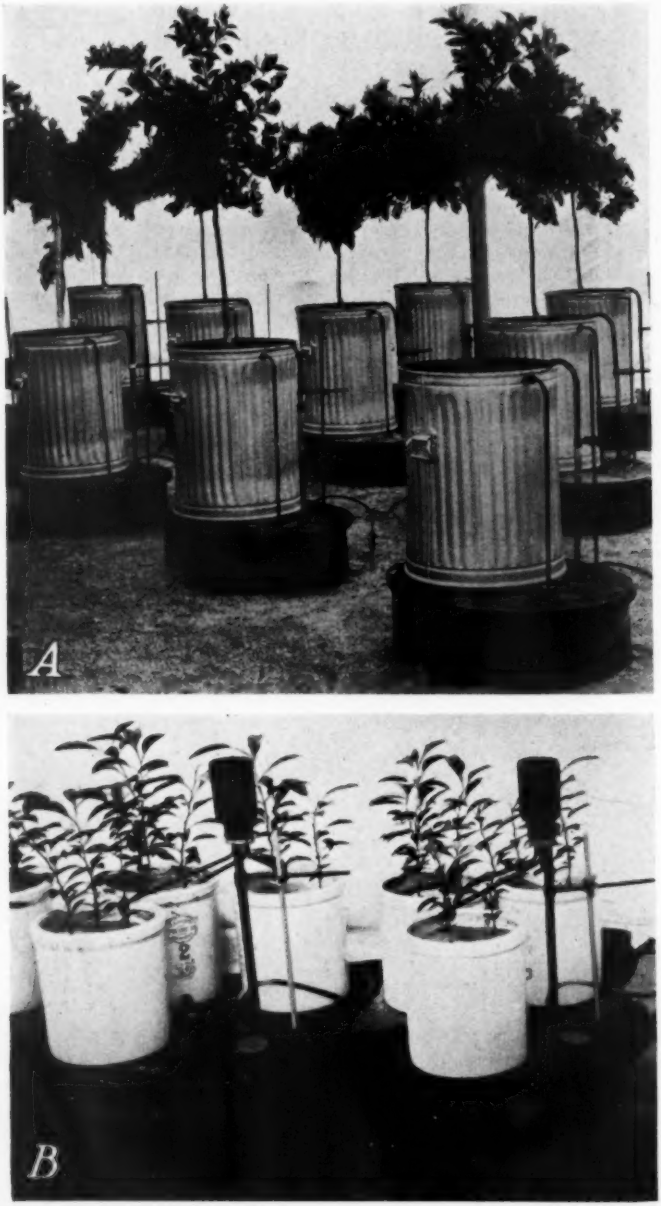

FIGURE 1.—Portion of outdoor (*A*) and indoor (*B*) automatic circulating sand-culture installations.

should discharge at a distance of about 2½ inches above the open end of the ejector tube.

The air pressure required to lift nutrient solution by this method is low, being just slightly in excess of that equivalent to the submergence

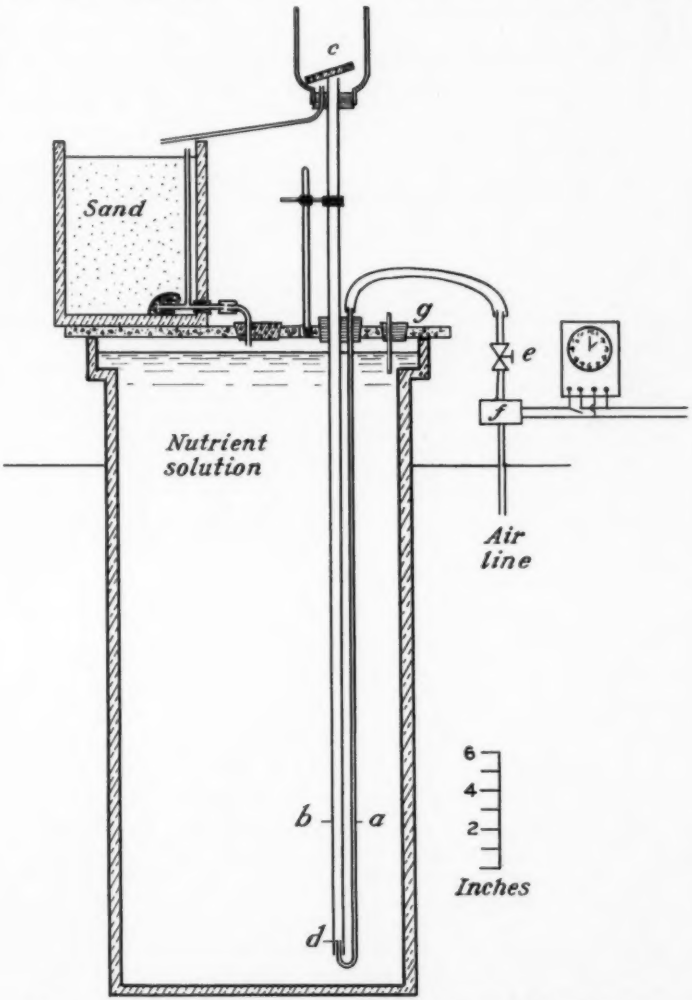

FIGURE 2.—Diagram of indoor unit: *a*, Air inlet tube; *b*, ejector member; *c*, distributor; *d*, point at which air enters *b*; *e*, needle valve; *f*, magnetic valve; *g*, volume indicator.

depth of the ejector tube. The efficiency of pumping depends upon the proportion of the total length of the ejector tube that is submerged. It is obvious that a reservoir cannot be pumped dry by this method. For best results, the submergence depth should be 50 percent or greater.

The rate of pumping can be regulated to some extent by the rate of air flow, and an air valve is required for each unit. (Needle valves are advantageous, but ordinary laboratory air cocks will suffice.) Losses of culture solution from the distributor (*c*) caused by the slight spouting nature of the discharge can be prevented by placing a free-moving cork of large diameter in this vessel.

The rate of pumping should be rapid enough to saturate the sand quickly and to establish free solution above the surface, thus assuring uniform displacement of the solution held by the sand. Overflows must be provided, as it is difficult to adjust the rate of pumping to the rate of percolation through the sand since the latter rate changes somewhat with temperature and stage of plant growth. By trials from time to time, the length of pumping time required to completely replace the old solution held by the sand can be determined. With the particular equipment here described, a 5-minute period for the 2-gallon cultures and a 10-minute period for the 25-gallon cultures is ample.

The volume of air used in relation to the volume of solution pumped and the percentage of ejector submergence for the two types of sand cultures is shown in table 1. At 50-percent submergence a given volume of air will lift a little less than half as much solution. The compressor capacity required for any number of units of size comparable to those herein described can be calculated from these data. If it is desired to operate a large number of units, they can be divided into groups, each group being operated in rotation by a separate time switch and magnetic valve. In this way the size of compressor required may be substantially reduced.

TABLE 1.—*Volume of air used as related to the amounts of solution pumped and percentage of ejection-tube submergence*

Type of sand culture	Height solution pumped	Submergence of ejector tube	Pumping interval	Volume of air used	Volume of solution pumped
	Inches	*Percent*	*Minutes*	*Liters* [1]	*Liters*
2-gallon pot	20	55	5	20.8	10.55
25-gallon garbage can	36	50	10	65.0	28.00

[1] Measured with capillary tube flow meter.

TIME SWITCH AND MAGNETIC VALVES

A simple telecron-motored time switch (G. E. type T–27), previously described by Eaton,[3] has been found satisfactory. Off-and-on riders suitably spaced on a revolving dial make it possible to adjust the time interval between operations to as little as 1 hour and 45 minutes. The actual "on" period can be adjusted from an instant up to about 15 minutes with the "on" riders set at 1 hour and 45 minutes apart.

General controls type K–10 and K–20 magnetic solenoid valves have been in continuous use for over a year and no trouble has been experienced in operating them under air pressures varying from 5 to 30 pounds.

RESERVOIRS

The authors have found that glazed sewer pipes serve especially well as reservoirs. Choice of sizes, durability, low cost, and depth,

[3] EATON, F. M. See footnote 2.

the last named lending itself to efficient pumping by the air lifts, are some of the more important advantages of sewer pipes. For the indoor equipment, sewer pipes with glazed stoppers sealed into the small end were especially made by the manufacturer. In the outdoor equipment, suitable stoppers were sealed into the bell end of the sewer pipe by means of asphaltum [4] prepared for this purpose. Glazed sewer pipes are not entirely impervious, owing to imperfections in the glaze; hence the inside surface should be coated with hot asphaltum or a nontoxic asphalt paint.

Specially made concrete lids 1 inch thick served both as covers for the reservoirs and as supports for the sand cultures. These lids were reinforced with chicken wire and were provided with holes spaced to accommodate the airlift, drainage, and overflow tubes. Pieces of one-eighth inch galvanized-iron pipe screwed into couplings embedded in the cement, and provided with ring-stand clamps, afford a convenient means of support for the airlift.

DRAINS AND OVERFLOWS

With earthenware crocks having a side opening near the bottom, a glass T tube with an extended arm fitted through a tightly fixed rubber stopper, as shown in figure 2, provides for both drainage and overflow. A pad of glass wool over which is placed a cover glass to prevent excessive packing permits free drainage without loss of sand. With the 25-gallon garbage cans a short piece of three-eighths inch galvanized pipe soldered into a hole drilled in the bottom of the can and covered with several layers of graded quartz gravel has provided adequately for drainage. The overflows for these containers are shown in figure 1.

EXPERIMENTAL DATA

IRON SUPPLY

In work with sweet orange (*Citrus sinensis* (L.) Osbeck) seedlings and tomatoes, (*Lycopersicum esculentum* Mill.), the authors have found, in conformity with the experience of Eaton,[5] that finely divided magnetite incorporated with the quartz sand serves as an excellent source of iron when used with slightly acid culture solutions of low phosphate content. On the basis of the authors' experience, it appears likely that by using a sufficient quantity, this source of iron can be utilized under a much wider range of experimental conditions. With 0.1 percent of magnetite in sand containing 2.5 percent of calcium carbonate, cultures of sweet orange seedlings after a time began to display typical iron-deficiency chlorosis. However, when an additional 0.4 percent of magnetite was incorporated with the sand (the chlorotic plants having been temporarily removed), the yellowed leaves began to green up in about 10 days and healthy new shoots subsequently emerged. Control cultures receiving nutrient solution from the same reservoir but not receiving the additional magnetite remained chlorotic. Again, sweet orange seedlings grown in sand cultures containing 0.1 percent of magnetite and supplied with nutrient solution of pH 6.3 and a content of PO_4 maintained at

[4] G–K compound sold by the Atlas Mineral Products Corporation, Mertztown, Pa.
[5] Eaton, F. M. See footnote 2.

4 parts per million have shown no symptoms of iron deficiency. However, when the phosphate concentration was raised to 10 parts per million PO_4, some of the plants became chlorotic. By simply increasing the acidity from pH 6.3 to 5.0, the chlorosis disappeared. No doubt, as in the case with the calcium carbonate, an increase in the amount of magnetite would have accomplished the same result.

The authors have found that magnetite fixes phosphate. The aforementioned observations relative to the influence of the concentration of hydrogen ions and of phosphate on the availability of the iron of magnetite is no doubt in part related to phosphate fixation, as well as to the influence of acidity on iron solubility. However, iron availability within the plant is apparently also influenced by acidity and phosphate variations in the nutrient medium.[6] Since the iron is not rendered completely unavailable by virtue of its capacity to fix phosphate, it seems certain that the fixed phosphate must be partly available. The use of magnetite is therefore inadvisable in experiments where the influence of the phosphate level is to be studied. Although subject to this limitation, it appears likely that by utilizing a sufficient quantity of magnetite, this method of supplying iron can be satisfactorily employed under a wide range of experimental conditions. Owing to the variable feeding power and requirements of plants for iron, on the one hand, and the influence of varying nutritive conditions on iron availability, both within and without the plant, on the other, it is advisable to determine by preliminary experiment how much magnetite to incorporate with the sand.

FREQUENCY OF FLUSHING SAND CULTURES

Obviously no given routine can be followed in all cases with regard to the frequency with which cultures must be flushed to assure nutrient control in the root zone. The kind of plant, stage of growth, temperature, light, and humidity, and the moisture-holding capacity of the sand, all enter into this question. In experiments where small sweet orange seedlings were grown in solutions of maintained but very low nitrate levels (0.7 parts per million N as NO^3), three flushings a day (8 a. m., 1 p. m., and 5 p. m.) proved inadequate. When hourly flushings during the daylight hours were adopted, the nitrate-deficiency symptoms exhibited under the three-times-a-day routine disappeared, the plants becoming green and resuming growth. At low nitrate levels, this ion is apparently absorbed relatively more rapidly than water, and a dilution of the solution film is thereby caused in the immediate vicinity of the absorbing surface. Apparently diffusion of nitrate into this zone from points removed is not rapid enough to compensate for the absorption withdrawal. Inasmuch as this observation was made at a time when the citrus plants were small (4 to 5 inches high) and with a plant which grows and absorbs rather slowly, it is apparent that frequent circulation is necessary to maintain the concentration of ions present in great dilution.

Although citrus seedlings are fairly sensitive as regards oxygen requirements in the root medium, the authors have experienced no difficulty with root rotting in cultures flushed at hourly intervals

[6] OLSEN, C. IRON ABSORPTION AND CHLOROSIS IN GREEN PLANTS. Compt. Rend. Lab. Carlsberg, Ser. Chim. 21 (3): 1–52, illus. 1935.
WADLEIGH, C. H., ROBBINS, W. R., and BECKENBACH, J. R. THE RELATION BETWEEN THE CHEMICAL NATURE OF THE SUBSTRATE AND THE DEGREE OF CHLOROSIS IN CORN. Soil Sci. 43: 153–175, illus. 1937.

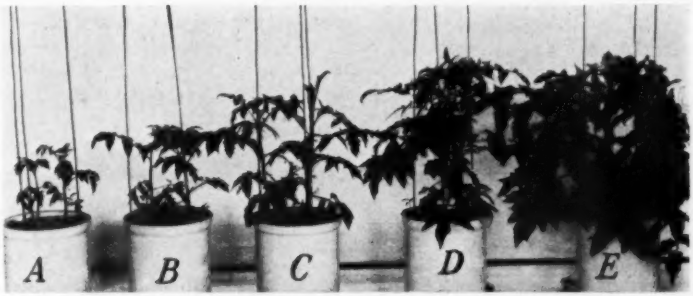

FIGURE 3.—Growth of tomatoes in 2-gallon sand cultures flushed at hourly intervals during daylight with solutions of graded nitrate levels. Maintained at indicated nitrogen levels (±15-20 percent) in parts per million: *A*, 0.14; *B*, 0.70; *C*, 1.4; *D*, 7.0; *E*, 70.0. Seed planted in pots July 3, 1936; plants photographed August 16, 1936.

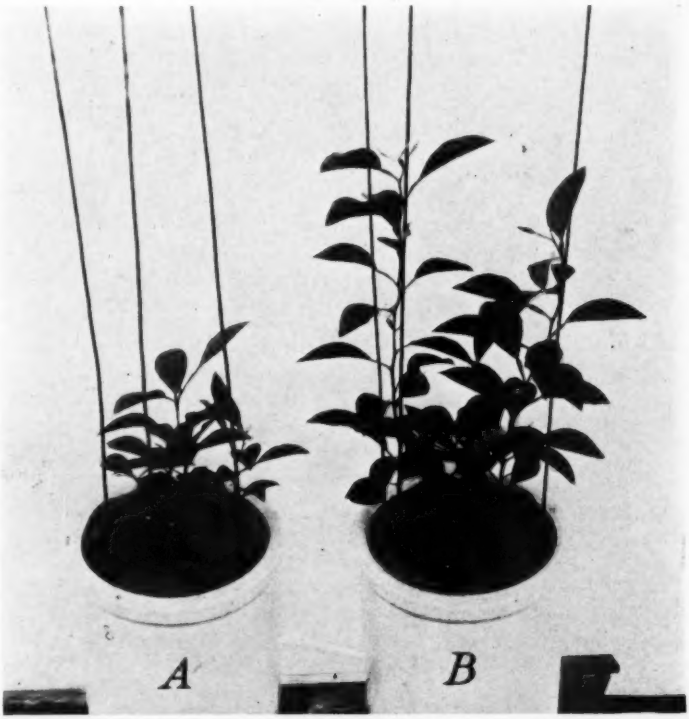

FIGURE 4.—Comparative growth of sweet orange seedlings in (*A*) a well-fertilized loam soil, and in (*B*) a sand culture supplied with a favorable culture solution. Plants in both soil and sand were deep green and healthy, but the growth rate in soil was much slower.

during the 24-hour period, this routine having been followed for months at a time. Obviously, as the excess solution drains from the sand, air takes its place, thus providing effective aeration. The air-lift method of pumping also tends to keep the solution well aerated.

Although compressed air contains traces of oil picked up from the compressor, no evidences of injury to the plants have been noted.

TYPE OF RESULTS SECURED

Both citrus seedlings and tomato plants have made excellent growth in cultures flushed at hourly intervals during the daylight period. Figure 3 shows some results with tomatoes grown in solutions of various nitrate levels.

The comparative growth of sweet orange seedlings during a 3-month period in a well-fertilized loam soil and in a sand culture flushed at hourly intervals during daylight is shown in figure 4. The plants in both the soil and sand were green and healthy, but the growth rate in the soil was much slower than that in the sand.

SUMMARY

Details of two automatically operated sand-culture installations are presented. The nutrient solution is circulated by means of compressed air-operated ejector-type pumps. A clock-controlled magnetic valve in the air line provides for periodic and automatic pumping. This system has given satisfactory service over long periods with little attention other than that required for the maintenance of nutrient-solution concentration. Various experiences relative to iron supply and frequency of flushing are given, together with examples of the excellent results capable of being secured with this type of equipment.

U. S. GOVERNMENT PRINTING OFFICE: 1938